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(54) Title: BIOPOLYMER SYNTHESIS UTILIZING SURFACE ACTIVATED, ORGANIC POLYMERS

(57) Abstract

Disclosed herein are surface activated, organic polymers useful for biopolymer synthesis. Most preferably, aminated polypropylene is used for the synthesis of oligonucleotides thereto, and these devices are most preferably utilized for genetic analysis of patient samples.

BIOPOLYMER SYNTHESIS UTILIZING
SURFACE ACTIVATED, ORGANIC POLYMERS

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10

FIELD OF THE INVENTION

15   The present invention is directed to the
synthesis of biopolymers or biomonomers onto, and the
attachment of previously synthesized biopolymers onto,
surface activated, organic polymers. The present
invention finds particular usefulness in the synthesis of
20   nucleic acids, peptides, proteins, as well as sequencing
by hybridization, peptide/protein sequencing, and
diagnostic evaluation at the genetic level.

BACKGROUND OF THE INVENTION

25

          Articles and publications set forth in this
patent disclosure are presented for the information
contained therein; none of this information is admitted
to be statutory "prior art" and we reserve the right to
30   establish prior inventorship with respect to any such
information.

          Deoxyribonucleic acid ("DNA") and ribonucleic
acid ("RNA") are long, threadlike macromolecules, DNA
35   comprising a chain of deoxyribonucleotides, and RNA
comprising a chain of ribonucleotides. A nucleotide
consists of a nucleoside and one or more phosphate
groups; a nucleoside consists of a nitrogenous base
linked to a pentose sugar. Typically, the phosphate
40   group is attached to the fifth-carbon ("C-5") hydroxyl
group ("OH") of the pentose sugar; however, it can also
be attached to the third-carbon hydroxyl group ("C-3
OH"). In a molecule of DNA, the pentose sugar is

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deoxyribose, while in a molecule of RNA, the pentose
sugar is ribose. The nitrogenous bases in DNA are
adenine ("A"), cytosine ("C"), guanine ("G"), and thymine
("T"). These bases are the same for RNA, except that
5   uracil ("U") replaces thymine. Accordingly, the major
nucleosides of DNA, collectively referred to as
"deoxynucleosides" are as follows: deoxyadenosine ("dA");
deoxycytidine ("dC"); deoxyguanosine ("dG"); and
thymidine ("T"). The corresponding ribonucleosides are
10 designated as "A"; "C"; "G"; and "U". (By convention,
and because there is no corresponding thymidine
ribonucleoside, deoxythymidine is typically designated as
"T"; for consistency purposes, however, thymidine will be
designated as "dT" throughout this disclosure).

15
The sequence of the nitrogenous bases of the
DNA or RNA molecule encodes the genetic information
contained in the molecule. The sugar and phosphate
groups of a DNA or RNA molecule perform a structural
20 role, forming the backbone of the molecule.
Specifically, the sugar moiety of each nucleotide is
linked to the sugar moiety of the adjacent nucleotide
such that the 3'-hydroxyl of the pentose sugar of one
nucleotide is linked to the 5'-hydroxyl of the pentose
25 sugar of the adjacent nucleotide. The linkage between
the two pentose sugars is typically via a phosphodiester
bond. Based upon this linkage protocol, one end
("terminus") of the nucleotide chain has a 5'-terminus
(e.g. hydroxyl, phosphate, phosphates, etc.), and the
30 other end has e.g., a 3'-hydroxyl or phosphate group. By
convention, the base sequence of a nucleotide chain is
written in a 5' to 3' direction, i.e., 5'-ATCG-3', or,
simply ATCG.

35
DNA and RNA are produced internally by living
animals; however, DNA and RNA can be chemically
synthesized such that synthetic strands of DNA and RNA

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can be rapidly and efficiently produced. These strands
are typically referred to as "synthetic oligonucleotides"
or "oligonucleotides." A widely utilized chemical
procedure for the synthesis of oligonucleotides is
5   referred to as the "phosphoramidite methodology." See,
e.g., U.S. Patent No. 4,415,732; McBride, L. and
Caruthers, M. Tetrahedron Letters, 24:245-248 (1983); and
Sinha, N. et al. Nuc. Acids Res. 12:4539-4557 (1984),
which are all incorporated herein by reference.
10   Commercially available oligonucleotide synthesizers based
upon the phosphoramidite methodology include, e.g., the
Beckman Instruments OLIGO 1000; the Millipore 8750™; and
the ABI 380B™, 392™ and 394™ DNA synthesizers.
Regardless of the protocol or the instrument, most
15   typically synthetic oligonucleotides are "grown" on a
support material, typically referred to as a "solid
support". Solid supports are varied and well-known;
specifics regarding solid supports will be set forth in
detail below.
20

       The importance of chemically synthesized
oligonucleotides is principally due to the wide variety
of applications to which oligonucleotides can be
directed. For example, oligonucleotides can be utilized
25   in biological studies involving genetic engineering,
recombinant DNA techniques, antisense DNA, detection of
genomic DNA, probing DNA and RNA from various systems,
detection of protein-DNA complexes, detection of site
directed mutagenesis, primers for DNA and RNA synthesis,
30   primers for amplification techniques such as the
polymerase chain reaction, ligase chain reaction, etc,
templates, linkers, and molecular interaction studies.
Recent attention in the area of oligonucleotide synthesis
has focused on procedures generally referred to as
35   Sequencing by Hybridization ("SBH"), as first disclosed
by Edwin Southern (see European Patent Application No. WO
89/10977, "Analyzing Polynucleotide Sequences").

The primary repeating structures of DNA and RNA molecules can be depicted as the following nucleosides:

DNA

RNA

5

B= adenine, thymine
guanine, cytosine

B_1= adenine, uracil
guanine, cytosine

10
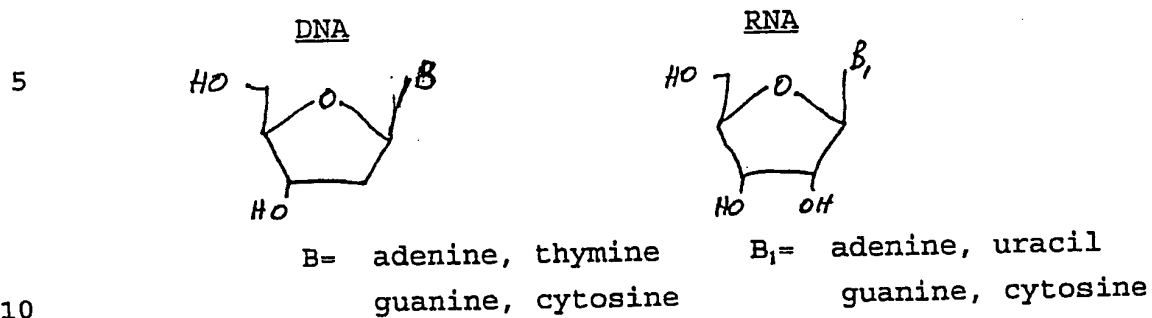
The key step in nucleic acid synthesis is the specific and sequential formation of internucleotide phosphate linkages between a 5'-OH group of one nucleotide and a

15 3'-OH group of another nucleotide. Accordingly, in the typical synthesis of oligonucleotides, the phosphite group of an "incoming" nucleotide is combined with the 5'-OH group of another nucleotide (i.e. the 5'-OH group is "phosphorylated" or "phosphitylated"). These groups

20 must be capable of actively participating in the synthesis of the oligonucleotides. Thus, the 5'-OH groups are modified (typically with a dimethoxy trityl ("DMT") group) such that an investigator can introduce two such nucleotides into a reaction chamber and adjust

25 the conditions therein so that the two nucleotides are properly combined; by a series of successive such additions, a growing oligonucleotide having a defined sequence can be accurately generated.

30 Proteins and peptides are essential components of all living cells. They are the structural elements of cell walls and cell membranes, enzymes, immunoglobulins, antibodies, transport molecules and most hormones. The building blocks of proteins and peptides are the twenty

35 natural amino acids. Each amino acid is "encoded" by the sequential grouping of three nucleotides, referred to as a "codon". Because there are four different nucleotides

and three nucleotides are required to encode an amino acid, there are 64 possible codons (4^3). Thus, several codons can encode for the same amino acid; for example, the codons GCG, GCA, GCT and GCC all encode for the amino

5   acid alanine.

A series of amino acids correctly linked together via amide bonds form protein chains, and the amino acid sequence of such a protein chain ("primary

10 structure") determines the very complex secondary and territory structures responsible for the biological functions of the proteins.

Each amino acid has an amino and carboxyl

15 terminal, such that proteins and peptides have an amino ("N-") and a carboxyl ("C-") terminal end. The general formula of an amino acid can be depicted as follows:

20
$$H_2N - \overset{\displaystyle \overset{H}{|}}{\underset{\displaystyle \underset{R}{|}}{C}} - COOH$$
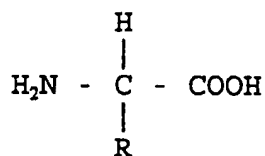
25 where R is one of at least 20 different side chains (for example, the side chain for alanine is a CH_3 group). The "NH_2" group is the amino group, and the "COOH" group is the carboxyl group.

30   As with nucleic acids, synthetic linear or branched amino acid chains can be chemically synthesized. A particularly well known procedure for the synthesis of linear amino acid chains, referred to as "solid phase peptide synthesis", was introduced by Merrifield in 1963.

35 See generally, Barany, G. and Merrifield, R. B. (1980) in The Peptides, 2:1-284. Gross, E. and Meienhofer, J. Eds. Academic Press, New York. Automated peptide synthesizers which utilize solid phase peptide synthesis protocols

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include, for example, the ABI 430™ and 431™, the
Millipore 9050 Plus PepSynthesizer™, and the Milligen
9500™ and 9600™. Typically, the C-terminal end of the
first amino acid is coupled to a solid support comprising

5   a reactive group (i.e., the site of attachment), while
the N-terminal end of the first amino acid is protected
with a labile protecting group (i.e., a group that can be
readily removed). The side chain functional groups on
the amino acids must be protected with "temporary"

10   protecting groups. Under appropriate conditions, a
similarly protected amino acid is added to the
insolubilized first amino acid which has had the labile
protecting group removed therefrom. By a series of
successive additions, an amino acid chain can be

15   synthesized, the final step typically being the cleavage
of the chain from the solid support and the removal of
the temporary protecting groups from each amino acid side
chain. This leads to a biologically active protein or
peptide.

20

       Oligosaccharides are the building blocks for
glycopeptides and glycolipids; glycopeptides and
glycolipids can be important mediators of biological
activity by interacting with cell membrane surfaces.

25   Thus, synthetic oligosaccharides can be utilized, inter
alia, to target specific cell membrane surfaces or to
interfere with the natural binding of glycopeptides and
glycolipids to a cell membrane surface. Synthetic
oligosaccharides have recently gained notoriety for their

30   ability to target a specific drug to a specific tissue.
The solid phase synthesis of oligosaccharides has been
reported using poly (ethylene glycol) monomethyl ether as
the solid support. See, Douglas, S.P. et al. J. Am.
Chem. Soc. 113: 5095-5097 (1991). See also, Rudemacher,

35   T.W. et al. "Glycobiology" Ann. Rev. Biochem. 57: 785-838
(1988).

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The solid supports utilized for, _inter alia_, nucleic acid, protein/peptide, and oligosaccharide synthesis are varied. With respect to nucleic acid synthesis, a widely utilized solid support material is controlled pore glass ("CPG"). See, for example, United States Patent No. 4,458,066. Other materials include nylon, polystyrene, polyacrylamide and cellulose. Teflon™ fiber support has been described as a substrate for oligonucleotide synthesis. See, Lohrmann, R.A. and Ruth, J. (1984) _DNA_ _3_:122; PCT Publication WO 85/01051 (published: March 14, 1985); and Molecular Biosystems, Inc. Oxidizable Solid Supports (Cat. No. OSS-01 and OSS-02). With respect to protein/peptide synthesis, such materials include, for example, cross-linked polystyrene, cellulose and polyamide resins. United States Patent No. 4,923,901 describes modified membranes having bound thereto oligonucleotides and peptides. As noted, poly (ethylene glycol) monomethyl ether has been used as a solid support for oligosaccharide synthesis.

An ongoing need exists for solid supports useful in the synthesis of these types of materials. This is because the materials heretofore utilized have associated drawbacks. For example, certain supports require the use of "spacer arms" or linkers to, in effect, couple the amino acids or proteins/peptides to the solid support; typically, when such linkers are utilized, it is often necessary to block sites on the membrane where the linkers are not located, in an effect to decrease or prevent non-specific binding of the biomonomers and biopolymers to the "non-linker" locations on the support. See, for example, Zhung, Y., et al. "Single-base mutation analysis of cancer and genetic diseases using membrane bound modified oligonucleotides" _Nuc. Acids Res._ _19(14)_:3927-3933 (1991)(nylon). Other materials require the use of surface modification to graft onto the surface of the solid support an

appropriate material which can in turn bind the
biomonomers and biopolymers. See, for example, United
States Patent No. 4,923,901 (polypropylene). Still other
materials require, for example, chemical modification of
5   the support to provide the necessary linkages between the
support and the biomonomers and biopolymers. See, for
example, United States Patent No. 4,458,066 (inorganic
polymers). As is evident, these additional steps add the
potential for errors, and hence can negatively impact
10   upon positive analytical results, as well as
significantly increasing the cost of the support.

What are needed, and hence, what would
contribute to the state of the art, are materials which
15   can be used for the synthesis of oligonucleotides and
proteins/peptides which do not require such additional
protocols such that the material is capable of being
rapidly, efficiently and economically prepared. With an
appropriate solid support method, oligonucleotides could
20   be synthesized thereon, and the resulting product
utilized for the analysis of patient sample DNA for
determination of presence or absence of specific genetic
mutations.

25   **SUMMARY OF THE INVENTION**

The present invention satisfied these needs by
providing organic polymers useful in the synthesis of
biopolymers, the organic polymers being modified with an
30   external chemical species, the modification being
accomplished by means of the application of energy in the
microwave or radio-frequency bands to the organic polymer
in the presence of the external chemical species. In
particularly preferred embodiments, oligonucleotides
35   complementary to regions of genes of interest are
synthesized onto the polymers, and these are in turn

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utilized for the analysis of patient samples for the
presence or absence of particular genetic mutation(s).

Preferably, the polymers are chemically inert
under conditions appropriate for biopolymer synthesis and
comprise a carbon backbone comprising various elemental
substituents, including, but not limited to, hydrogen,
carbon, oxygen, fluorine, chlorine, bromine, sulphur and
nitrogen. Representative polymers include, but are not
limited to, polypropylene, polyethylene, polybutylene,
polyisobutylene, polybutadiene, polyisoprene,
polyvinylpyrrolidone, polytetrafluoroethylene,
polyvinylidene difluoride, polyfluoroethylene-propylene,
polyethylenevinyl alcohol, polymethylpentene,
polychlorotrifluoroethylene, polysulfones, and blends of
copolymers thereof. Polypropylene is a particularly
preferred polymer.

Preferably, the external chemical species is:
(1) a chemical species not previously adsorbed to the
surface of the polymer; and (2) becomes a nucleophile
when adsorbed to the surface of the polymer, preferably
via an elevated energy state. Most preferably, the
elevated energy state is a radio-frequency plasma
discharge, a microwave frequency plasma discharge, or a
corona discharge.

Preferably, biopolymers include, but are not
limited to, nucleic acids, proteins, peptides, hormones,
oligosaccharides, lipids or the synthetic analogues
thereof, such as inverted nucleotides (Ortigâo, J. et al.
Antisense Res. Dev. 2:129 (1992)), peptide nucleic acids
(Egholm M. et al. *J. Am. Chem. Soc.* 114:1895 (1992)) and
Meta-DNA (Hashimoto, H. & Switzer, C. *J. Am. Chem. Soc.*
114:6255 (1992)), and combinations of the above. The
biopolymer synthesis is preferably accomplished by
organic, inorganic, or biochemical means, and

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combinations thereof. Most preferably, oligonucleotides
are synthesized onto the polymers, and, as noted, these
are utilized for analysis of patient samples comprising
DNA.

5

      In a particularly preferred embodiment of the
invention, the organic polymer is polypropylene, the
external chemical species are nitrogen and hydrogen (in
the form of an ammonia gas), the nucleophile is an amine,
10   and the elevated energy state is achieved via radio
frequency plasma discharge. Thus, a particularly
preferred surface activated organic polymer is a
polypropylene material aminated via radio frequency
plasma discharge; such materials are preferably utilized
15   for the "in-place", or "in-situ", attachment of
nucleotides and/or amino acids thereto, without the need
for spacer arms or linkers, and thus particularly well
suited for the synthesis of oligonucleotides and/or
peptides. The amine groups on the activated organic
20   polymers are reactive with nucleotides such that the
nucleotides and/or amino acids introduced thereto are
covalently attached onto the surface of the polymer.

      Such surface activated organic polymers find
25   particular utility in the areas of, e.g.,
oligonucleotide, peptide, oligosaccharide, and lipid
synthesis. With respect to, e.g., oligonucleotides
synthesized onto such polymers, these materials find
particular utility in the areas of, e.g., Sequencing by
30   Hybridization ("SBH") and genetic analysis for purposes
of medical and diagnostic evaluation. Because
polypropylene is "chemically inert", problems associated
with non-specific binding are substantially avoided such
that detection sensitivity is significantly improved.

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BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is the result of High Pressure Liquid Chromatographic analysis of a cleaved 17-mer oligonucleotide (with 5'-DMT protecting group) previously synthesized directly onto aminated polypropylene;

Fig. 2 is the result of Capillary Gel Electrophoresis analysis of the 17-mer oligonucleotide of Fig. 1, with the DMT group having been removed prior to analysis;

Fig. 3 is the compilation of dehybridization analyses of three oligonucleotide probes from a defined Target Oligonucleotide, the Target Oligonucleotide having been synthesized directly onto aminated polypropylene, each of the probes having various sequences which are complementary or non-complementary to the Target;

Fig. 4 is the compilation of dehybridization analyses of amplicons from CTFR Exon 10, Normal and CTFR Exon 10, ΔF508, against Target Oligonucleotide complementary to CTFR Exon 10, Normal, the Target Oligonucleotide having been synthesized directly onto aminated polypropylene; and

Fig. 5 is a laser-printer reproduction of a hybridization between a fluorescent-labelled probe and a target synthesized directly onto aminated polypropylene, with detection of the label via a CCD camera.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The solid phase synthesis of biopolymers (e.g., oligonucleotides, peptides, oligosaccharides, lipids, etc.) requires, by definition, a solid support material from which the initial starting material is attached and

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from which the synthesis of the biopolymer is initiated.
As has been noted, "The unique feature of solid phase
synthesis is the solid support itself, and future
improvement of the synthesis will probably depend upon

5   finding better supports". Wallace, R. B. and Itakura, K.
"Solid phase synthesis and biological applications of
polydeoxyribonucleotides" Chpt. 13, Solid Phase
Biochemistry Scouten, W.H., Ed. John Wiley & Sons (1983).
As those in the art will appreciate, this statement has

10   proven to be correct. As the chemistry involved in the
synthesis of biopolymers has improved; as the need for
such biopolymers, particularly oligonucleotides and
peptides, has increased; and as the area of application
of such biopolymers has expanded, the need for "better

15   supports" has indeed increased.

In essence, the benefits associated with
previous solid support strategies are typically also
directly related to the types and number of problems

20   occasioned by the use thereof. This is because to the
degree that such materials are conducive to chemical
interactions between the support material itself and the
biopolymer, by that same degree the materials can
interact in a non-specific manner with other materials.

25   For example, nylon-based filters find wide-spread
application in the area of DNA analysis whereby
oligonucleotides are cross-linked directly to the nylon.
However, nylon is very reactive with other materials such
that it is typically necessary, if not essential, to

30   chemically block any site on the nylon to which an
investigator does not wish to have such "non-specific"
materials bind thereto. As noted in the above-referenced
Zhung article, in an effort to attempt to prevent the
non-specific attachment of sample DNA to the location on

35   the nylon membranes where amino-linker bound
oligonucleotide probes were not located, it was necessary
to block these highly reactive sites to avoid non-

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specific binding thereto. This protocol was examined in
an effort to improve upon a previous procedure utilizing
poly-T tails as a linker between a solid support and
oligonucleotides. See, Saiki, R. K., et al (1989) PNAS
USA 86:6230-6234. It is noted that in these procedures,
the oligonucleotides are described as being synthesized
"off-line" and subsequently attached to the support via
an amino-linker or a poly-T tail.

        In the case of "on-line" (in-situ) synthesis,
the types of chemical manipulations that are required to
be utilized in conjunction with the available solid
supports are staggering. For example, in the case of
inorganic solid supports, such as CPG, silica, glass,
etc., the chemical structure of these materials creates a
"rigidity" which is believed to constrain the synthesis
of biopolymers. Thus, particularly in the case of CPG,
it is typically necessary to utilize chemical linkers in
conjunction therewith. In essence, these linkers,
regardless of their chemical composition or length, are
intended to provide a degree of "freedom" in the
synthesis by providing a chemically "flexible" moiety
which is attached to the support at one end and is
capable of binding to the growing biopolymer. Without
the linker, one can theoretically synthesize biopolymers,
but overall yields, the rapidity of synthesis, etc. are
jeopardized. As a further example of the use of
inorganic materials which utilize linkers, European
Patent Application No. WO 89/10977 discloses the use of
glass plates having aliphatic linkers bound thereto for
use in the in-situ synthesis of oligonucleotides thereon.

        As those in the art will appreciate, linkers
are very unpredictable. Stated again, it is not
necessarily apparent which type of linker, or the lengths
thereof, will be optimal for any given support material.
Thus, it is typically necessary to optimize the

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conditions, composition and length of any given linker
with any particular solid support that requires a linker.

5   Other supports are, in effect, not utilized as
supports for the purpose of biopolymer synthesis.
Rather, these materials are utilized for their ability to
chemically react with other materials which in turn are
conducive to such synthesis. For example, Khrapko, K.
R., et. al. "Method for DNA sequencing by hybridization
10   with oligonucleotide mixture" DNA Sequence - J. DNA
Sequencing and Mapping 1: 375-388 (1991), describe the
use of an inorganic material (glass) having a
polyacrylamide gel overlaid thereon such that previously
synthesized oligonucleotides can be covalently linked to
15   the polyacrylamide gel layer, but not on the inorganic
material. Thus, the glass apparently functions
principally as a form-support for the actual synthesis
support. Similarly, in United States Patent No.
4,923,901, chemically inert polymers such as
20   polypropylene and polyethylene are described as being
useful for nucleic acid and peptide synthesis, when
polymer monomers with applicable functional groups are
grafted onto the surface thereof.

25   In essence, the current state of the art runs
the gamut from highly reactive materials (such as nylon)
to materials which, in essence, serve as mere vehicles
for linkers (i.e. glass plates).

30   In terms of an overall objective of biopolymer
synthesis, a significant amount of energy and resources
has been expended in the development of more efficient
linkers or mechanisms for attaching one material to
another. See, for example, Maskos, U. & Southern, E. M.
35   "Oligonucleotide hybridizations on glass supports: a
novel linker for oligonucleotide synthesis and

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hybridization properties of oligonucleotides synthesized
in situ".   Nuc. Acids Res. 20(7): 1079-1684 (1992).

Recognizing the correctness of Wallace and
Itakura's focus on "better supports," we have embarked
upon an altogether different approach and have focused
our attention on the support material in toto. In so
doing, we recognize that a truly superior solid support
for use in biopolymer and biochemical synthesis is most
preferably a material that: lacks the chemical
structural rigidity of inorganic materials such that
linkers can be utilized, but are not required to be used
in conjunction with the support; is amenable to surface
activation such that upon activation, the surface of the
support is capable of covalently attaching biopolymers
thereto and, in conjunction with the first criteria, is
amenable to the in situ synthesis of such biopolymers; is
chemically inert such that at the conclusion of
biopolymer synthesis, regions on the support not occupied
by the biopolymers are not amenable to non-specific
binding, or when such non-specific binding does occur,
these materials can be readily removed from the surface
without removal of the biopolymer; and, is amenable to
ease of handling and manipulation such that the material
can be utilized in a variety of different contexts.

These criteria are accomplished by the use of
surface activated, organic polymers which are preferably
chemically inert under conditions conducive to biopolymer
synthesis.

As used herein, the term "organic polymer" and
"polymer" are intended to mean a support material which
is most preferably chemically inert under conditions
appropriate for biopolymer synthesis, and which comprises
a carbon backbone comprising various elemental
substituents including, but not limited to, hydrogen,

carbon, oxygen, fluorine, chlorine, bromine, sulphur and
nitrogen. Representative polymers include, but are not
limited to, polypropylene, polyethylene, polybutylene,
polyisobutylene, polybutadiene, polyisoprene,
5   polyvinylpyrrolidone, polytetrafluoroethylene,
polyvinylidene difluoride, polyfluoroethylene-propylene,
polyethylenevinyl alcohol, polymethylpentene,
polychlorotrifluoroethylene, polysulfones, and blends of
copolymers thereof. Most preferably, the polymer is
10   polypropylene.

As used herein, the term "surface activated"
when used in conjunction with polymer is intended to mean
the process of modifying a polymer such that external
15   chemical species become adsorbed onto the surface of the
polymer, whereby the chemical species are capable of
chemically linking biopolymers and biomonomers to the
surface of the polymer. Preferably, the chemical linking
is via a nucleophile, and most preferably the nucleophile
20   is on the surface of the modified polymer.

As used herein, the term "medium" when used in
conjunction with the term "polymer" is intended to mean
the physical, structural shape of the polymer; thus,
25   "medium" can be generally defined as polymer films (i.e.,
polymers having a substantially non-porous surface);
polymer membranes (i.e., polymers having a porous
surface); polymer filaments (e.g., mesh and fabrics);
polymer beads; polymer foams; polymer frits; and polymer
30   threads. Most preferably, the polymer medium is a thread
or a membrane or a film.

As used herein, the phrase "device-medium" when
used in conjunction with the term "polymer" is intended
35   to mean any device to which a polymer medium can be
affixed such as, microtiter plates, test tubes, inorganic
sheets, dipsticks, etc. For example, when the polymer

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medium is a polypropylene thread, one or more
polypropylene threads can be affixed to a plastic
dipstick-type device, or polypropylene membranes can be
affixed to glass slides. The particular device is, in

5   and of itself, unimportant -- all that is necessary is
that the polymer medium can be affixed thereto without
affecting the functional behavior of the polymer or any
biopolymer adsorbed thereon, and that the device intent
is stable within any materials to which the device is

10   introduced (e.g., clinical samples, etc.).

As used herein, the term "adsorbed" is intended
to have a meaning ordinarily ascribed thereto in the
chemical and biochemical arts. Stated again, a first

15   material that is adsorbed onto the surface of another
material becomes, in effect, a "part" of that material
such that the first material is not capable of being
easily removed from the surface of the other material.
For example, a surface activated polymer comprises

20   nucleophiles on the surface thereof; under appropriate
conditions, biomonomers, e.g., that react with the
nucleophiles will be covalently attached, and therefore
adsorbed, to the surface of the biopolymer via such
nucleophiles.

25

As used herein, the term "surface" is intended
to mean a depth of no more than about 5000 angstroms (Å),
preferably between about 10 and about 1000Å.

30   As used herein, the term "nucleophile" is a
chemical species comprising a pair of electrons which are
capable of combining with an electron-deficient species.
Preferably, the external chemical species becomes a
nucleophile when adsorbed to the surface of the polymer.

35   An "external chemical species" is a chemical species not
previously adsorbed to the surface of the polymer.
Stated again. an external chemical species becomes a

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nucleophile when it is adsorbed to the surface of the
polymer. Preferably, an external chemical species is
amenable to a plasma process. Typically, the plasma
process will create ionized and radical forms of the
external chemical species. Preferably, the external
chemical species are selected from the group consisting
of: nitrogen; oxygen; sulfur; carbon; hydrogen; argon;
helium; and combinations comprising at least one of the
foregoing. Nucleophilic forms of these external chemical
species include, e.g., amine; hydroxyl; thiol;
carboxylate; and substituents comprising at least one of
the foregoing. When an external chemical species is
subjected to a plasma, ionized and radical form(s) of the
external chemical species result. As those in the art
appreciate, under appropriate conditions, such ionized
and radical form(s) can "chemically" interact with the
polymer, whereas the non-ionized and non-radical form(s)
thereof do not have a tendency to form chemical bonds
with the polymer.

          As used herein, the term "biopolymer" is
intended to mean repeating units of biological or
chemical moieties. Representative biopolymers include,
but are not limited to, nucleic acids, oligonucleotides,
amino acids, proteins, peptides, hormones,
oligosaccharides, lipids, glycolipids, lipopoly-
saccharides, phospholipids, synthetic analogues of the
foregoing, including, but not limited to, inverted
nucleotides, peptide nucleic acids, Meta-DNA, and
combinations of the above. "Biopolymer synthesis" is
intended to encompass the synthetic production, both
organic and inorganic, of a biopolymer. Related to a
bioploymer is a "biomonomer" which is intended to mean a
single unit of biopolymer, or a single unit which is not
part of a biopolymer. Thus, for example, a nucleotide
is a biomonomer within an oligonucleotide biopolymer, and
an amino acid is a biomonomer within a protein or peptide

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biopolymer; avidin, biotin, antibodies, antibody
fragments, etc., for example, are also biomonomers.
Additionally, as used herein, the term "initiation
biomonomer" or "initiator biomonomer" is meant to
5   indicate the first biomonomer which is covalently
attached via reactive nucleophiles to the surface of the
polymer, or the first biomonomer which is attached to a
linker or spacer arm attached to the polymer, the linker
or spacer arm being attached to the polymer via reactive
10   nucleophiles.

As used herein, "analogue" or a "synthetic
analogue" when used in conjunction with a biomonomer or a
biopolymer refers to natural and un-natural variants of
15   the particular biomonomer or biopolymer. For example, an
"antibody analogue" includes chimeric antibodies,
monoclonal antibodies, and antibody fragments; an "amino
acid analogue" includes beta-alanine; a nucleotide
analogue includes inosine and dideoxynucleotides. The
20   foregoing is not intended to be exhaustive but rather
representative.

As used herein, the phrase "reverse dot blot"
is meant to indicate a protocol whereby biopolymers are
25   attached to a solid support, and the presence (or
absence) of constituents in a sample material are
detected via the application, and subsequent interaction
(or non-interaction), of the sample to the biopolymers.
As used herein, the phrase "dot blot" is meant to
30   indicate a protocol whereby constituents in or from a
sample material are attached to a solid support and
biopolymer or biomonomer probes are applied thereto.

As used herein, the terms "mask" or "masking"
35   are meant to indicate an article or a process for
selectively blocking a biological, chemical or physical
reaction from occurring. As a means of illustration, a

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mask could include a screening mechanism that is placed
on top of an organic polymer prior to surface activation
such that those regions of the polymer that are masked,
or covered, by the screening mechanism are substantially
5   non-surface activated after the polymer has been
subjected to surface activation techniques, whereby only
those areas that were not masked are substantially able
to participate in, e.g., biopolymer synthesis. The terms
"mask" and "masking" are not intended to be exclusively
10   static, such that dynamic masking, i.e. a series of
masking steps are utilized to selectively synthesize a
variety of different biopolymers on the same activated
organic polymer, is also intended to fall within the
scope of this definition.

15

         As used herein, the phrase "recognition domain"
is meant to indicate a sequence of amino acids that are
recognized by a receptor on a cell surface.

20   As used herein, the phrase "bioreactive
peptide" is meant to indicate a peptide capable of
eliciting a response from a cell; for example, a peptide
which binds to a recognition domain on the surface of a
cell is a bioreactive peptide.

25
         As used herein, the term "seed" is meant to
indicate a cell which binds to a bioreactive peptide and
to which other similar cell may attach.

30   For convenience, the remainder of the
disclosure focuses on the use of the polymer
polypropylene, the chemical species ammonia, plasma
protocols and the synthesis of oligonucleotides and
peptides. It is to be understood that other polymers,
35   chemical species, energy activation modes, and
biopolymers are amenable to the invention disclosed
herein.

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Polypropylene is chemically very inert and hydrophobic; thus, polypropylene can be utilized in very corrosive environments. For example, polypropylene has good chemical resistance to a variety of mineral acids (e.g., hydrochloric acid), organic acids (e.g., formic acid, acetic acid), bases (e.g., ammonium hydroxide, potassium hydroxide), salts (e.g., sodium chloride), oxidizing agents (e.g., peracetic acid, iodine solutions) and organic solvents (e.g., acetone, ethyl alcohol, acetonitrile, etc.). Additionally, polypropylene provides low fluorescence background. Polypropylene has the following chemical structure:

$$-(H_2C - CH - CH_2)_n-$$
$$|$$
$$CH_3$$

This structure is not functional vis-a-vis biopolymer synthesis; i.e., in and of itself, one cannot synthesize oligonucleotides using polypropylene as a support material. Thus, in order for polypropylene to be useful for the synthesis of biopolymers, the surface thereof must be modified via the introduction of, for example, amino groups onto the surface. An efficient, rapid and economical method for introducing such amino groups onto the surface of a polypropylene medium is by use of plasma discharge in an ammonia or organic amine containing gas.

A "plasma" is most preferably an ionized gas which gains sufficient ionization energy from an electromagnetic field. It exhibits long range electromagnetic forces and becomes a conductor of electricity. Plasma consists of a mixture of electrons, atoms, positive and negative ions and neutral free radicals. The overall electrical charge of the plasma is neutral. Plasma energy sources include, but are not limited to, direct current, alternating current, radio

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frequency, microwaves, shock waves and lasers. Low
temperature plasma treatments include radio frequency
plasma discharge ("RFPD") microwave frequency plasma
discharge ("MFPD") and corona discharge ("CD"); such
5   treatments all typically affect only the surface of a
solid material to a depth of no greater than about 1000Å,
leaving the remainder of the material unmodified.

Surface interactions with plasmas usually fall
10   into three general classes of reaction possibilities:
(1) chemical species on the polymer surface can be
removed from the surface; (2) external chemical species
can be added to the surface of the polymer; or (3)
rearrangement of bonds can occur within the surface of
15   the polymer itself. It is possible that more than one of
these reactions will occur at the same time. The
principal differences between plasma discharge and
alternative surface modifications (such as ionization, or
α or β-irradiation, which usually penetrate deeply into
20   the bulk of the polymer, grossly affecting the bulk
characteristics thereof), are the greater chemical
flexibility that can be realized in terms of the choice
of reactant gas or mixtures for producing reactive
species to produce various surface states, and the lack
25   of secondary reactive species which can cause undesirable
side effects.

Polypropylene can be surface activated via the
introduction of amino groups thereto using RFPD, MFPD or
30   CD in ammonia gas or other suitable amine introducing
entities including, but not limited, to C_1 - C_{12} aliphatic
or cyclic amines which may be primary, secondary or
tertiary. The hydrocarbon chain can be straight chain,
branched, saturated or unsaturated, and one or more amino
35   groups can be attached to the hydrocarbon chain. Methyl
amine, alkylamine, ethylenediamine, diaminocyclohexane
are examples of such amines. Ammonia is most preferred.

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In the presence of a RFPD, MFPD or CD, the most probable mechanism for the attachment of amino groups to a medium is as follows:

$$\text{NH}_3 \ \& \ -(\text{H}_2\text{C} - \underset{\underset{\text{CH}_3}{|}}{\text{CH}} - \text{CH}_2)_n- \ \overset{\text{RFPD}}{\longrightarrow} \ \overset{\bullet}{\text{N}}\text{H}_2 + \ -(\text{H}_2\text{C} - \underset{\underset{\text{CH}_3}{|}}{\overset{\bullet}{\text{C}}} - \text{CH}_2)_n-$$

$$\longrightarrow \ -(\text{H}_2\text{C} - \underset{\underset{\text{CH}_3}{|}}{\overset{\overset{\text{NH}_2}{|}}{\text{C}}} - \text{CH}_2)_n-$$
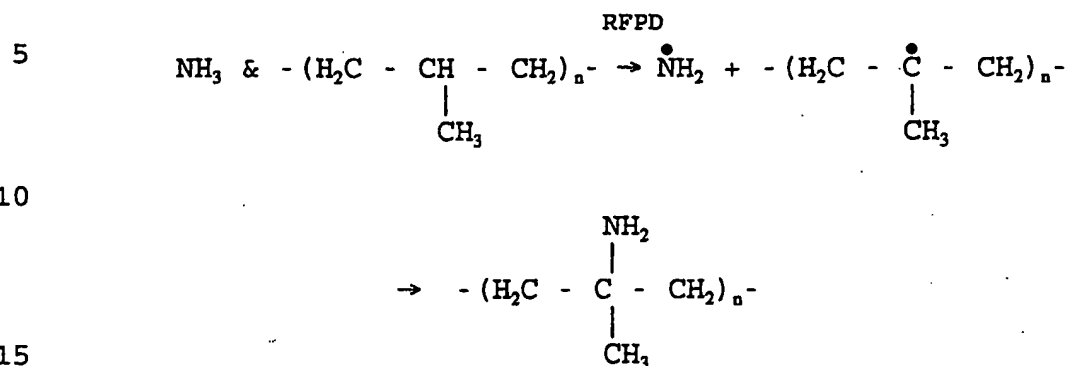
In the presence of oxygen radical, the resulting surface activated polypropylene comprises the following activated surface:

$$-(\text{H}_2\text{C} - \underset{\underset{\text{CH}_3}{|}}{\overset{\overset{\text{OH}}{|}}{\text{C}}} - \text{CH}_2)_n-$$

In the presence of sulfur radical, the resulting surface activated polypropylene comprises the following activated surface:

$$-(\text{H}_2\text{C} - \underset{\underset{\text{CH}_3}{|}}{\overset{\overset{\text{SH}}{|}}{\text{C}}} - \text{CH}_2)_n-$$
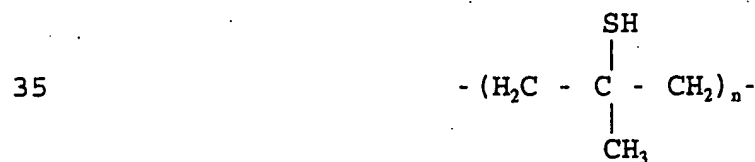
Thus, for example, using well known nucleic acid synthesis techniques, one can obtain the following adsorbed-initiation nucleotides:

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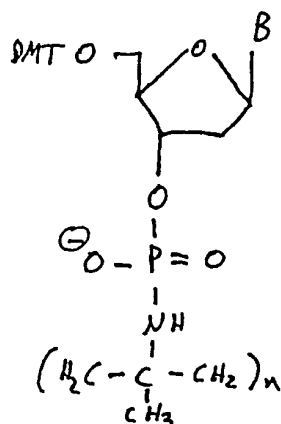
PHOSPHORAMIDATE   PHOSPHATE
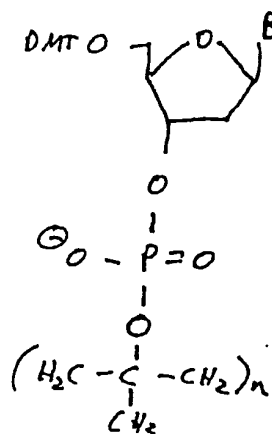
where "A" is a protecting group and "B" represents one of
the four bases. Because a chemical linker is not
required to attach the biomonomer to the polymer, the
initiation biomonomer is adsorbed, i.e., becomes, in
effect, a "part" of the polymer itself.

Beneficially, MFPD, RFPD and CD can be
efficiently controlled such that only a portion of the
polymer medium need be activated. Thus, by activating
only a portion of the surface, the remainder thereof
continues to be chemically inert, and therein lies a
benefit of the present invention. By activating only a
portion of (preferably) one side of the surface of the
polymer medium, only areas which are activated will be
amenable to biopolymer synthesis such that at the
conclusion of, e.g., _in situ_ synthesis of
oligonucleotides, substantially all of he remainder of
the surface will be chemically inert. Thus, if such
oligonucleotides are used as probes for a genetic trait,
problems associated with non-specific binding of nucleic
acid macromolecules to the surface are avoided.

Preferably, less than about 50 nmoles per
square centimeter ("nmoles/cm^2") of the surface of the
polymer medium comprises the activated chemical species,
and more preferably between about 5 to 15 nmoles/cm^2. It

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is to be understood that these values are with respect to
the height-by-width surface area of the polymer material;
thus, in a situation where the polymer material comprises
a porous surface, the foregoing values do not apply to
the total surface area which, given the surface area
attributed to the pores, is much greater than a height-
by-width surface area. Alternately, it is preferred that
less than about 15%, more preferably less than about 10%,
and most preferably less than about 5% of the surface of
the polymer medium comprise the activated chemical
species. While this area may seem "small", or the
percentages "low", these are relative values. For
instance, one could utilize a polymer medium having,
e.g., a 100% activated chemical species surface.
However, this could lead to problems in that all of the
activated species might not be occupied by an initiation
biomonomer and thus the potential for non-specific
binding increases. However, as the percentage of surface
activation decreases, the potential for ensuring that
each activated portion of the surface is occupied by an
initiation biomonomer substantially increases
(particularly because the surrounding area is chemically
inert).

Plasma generating devices are commercially
available and applicable to the invention. A
particularly preferred plasma generator is available from
Plasma Science, Foster City, CA (Model No. PS0150E radio-
frequency). Such devices are preferred because the
conditions for introduction of gases, power, time of
plasma discharge, etc. can be readily selected, varied,
optimized and controlled. These parameters can be
optimized with little experimentation, principally
because the physical condition of the polymer medium is
adversely affected if, for example, the amount of power
(typically in watts) is too high, or the length of time
of plasma discharge is too great -- such adverse affects

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are typically manifested by the creation of a "brittle"
polymer medium. Accordingly, to the degree necessary,
and consistent with the parameters disclosed herein,
those skilled in the art are credited with the ability to
5   optimize the conditions for efficient surface activation
of the polymer medium.

With respect to the amination of polypropylene,
it is preferred that the gas comprises the following
10   components in the following ranges: ammonia (about 99%
to about 100%) and oxygen (about 0 to about 1%).
Preferably, the wattage of the power supply is between
about 10 and about 500 watts, more preferably between
about 100 and about 400 watts, and most preferably about
15   200 watts. Preferably, the polypropylene and gas are
subjected to the plasma discharge for less than about 10
minutes, more preferably between about 1 and about 5
minutes, and most preferably about 2 minutes. With
respect to the type of plasma discharge, it is preferred
20   that radio frequency waves be utilized; preferably these
are within the range of from about 1 MHz to about 20 MHz,
and most preferably about 13 MHz. With respect to
microwave plasma discharge, it is preferred that the
microwaves be in the range of from about 1,000 MHz to
25   about 3,000 MHz, and most preferably about 2,000 MHz.
With respect to corona discharge, it is preferred that
the treatment power applied is between about 10 to about
250 watts, more preferably at the electrode between about
10,000 and 20,000 volts.
30

The polymer medium can be varied in accordance
with the needs of the investigator. It is preferred that
the polymer medium be amenable to dissection after
biopolymer synthesis; however, this preference is
35   principally based upon the intended uses of the
biopolymers synthesized thereon. In the area of, e.g.,
nucleic acid analysis at the genetic level, we consider

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it absolutely essential that the testing devices be
readily subjected to quality control procedures. By way
of analogy, in the area of pharmaceutical manufacturing,
it is relatively easy to segregate a portion (or

5   portions) of a large-scale lot of the pharmaceutical once
it is manufactured: if that portion(s) meets necessary
quality assurance and quality control ("QA/QC")
parameters, then, from a statistical viewpoint, the
entire lot is of that same quality; if, on the other

10   hand, that portion(s) fails the necessary QA/QC control
parameters, the entire lot is suspect. The principal
assumption behind this approach is that all of the
parameters involved in the production of that lot were
the same such that any one sample portion(s) should be

15   representative of the entire lot. As the arena of
genetic analysis expands and finds increasing
utilization, similar assurances will be demanded and
required.

20   It is with the foregoing in mind that a
preference is indicated for the polymer medium bearing
amenable to dissection after biopolymer synthesis.

              Such a feature allows for the ability to QA/QC
25   the biopolymer synthesized onto the polymer medium. For
example, in the case of membranes, a section or sections
thereof can be dissected therefrom such that detailed and
stringent analytical techniques can be readily applied
thereto -- if such analysis indicate that, e.g., a

30   particular oligonucleotide sequence is present, then from
a statistical perspective, the entire membrane has that
sequence.

              Dissection of the polymer medium also allows
35   for the generation of multiple media, each comprising the
defined biopolymer. These can then be dissected and one
piece from each medium can be affixed to a unitary

device-medium. Thus, for example, a plethora of
dipsticks can be readily created, each dipstick
comprising several different polymer media sections
comprising specific biopolymers; thus, the conditions for
5   synthesis of any particular biopolymer is identical and
such biopolymer is amenable to QA/QC protocols.

While the needs of the investigator will
determine the specific parameters involved in the
10 capability of dissection, we prefer to utilize, in the
case of membranes, polypropylene having a thickness of
between about 80 to about 100 μm, and in the case of
threads, polypropylene having a diameter of about 0.001
inches. In the case of membranes, dissection can be
15 readily accomplished with any severing device (e.g.,
scissors). In the case of threads, it is preferred that
these be dissected with a device which can "seal" the
ends at the location of the cut, i.e., a laser, an
ultrasonic device or a hot-knife; this preference is
20 predicated upon a practical concern, to wit, threads are
composed of entwined fibers such that when the thread is
cut, the fibers can become disassociated from each other.
(The fibers themselves can, of course, be utilized
directly, but threads are preferred because they are
25 somewhat easier to manipulate; however, this is, again, a
function of the desires of the investigator).

The disclosed polymers are particularly well
suited for the direct synthesis of, e.g., oligo-
30 nucleotides and peptides thereon. Beneficially, a
variety of commercially available nucleic acid
synthesizers are available, including the Beckman
Instrument OLIGO 1000. Focusing on aminated
polypropylene membranes or threads, immediately following
35 the amination process (or after removal from storage),
the materials can be incorporated directly into the
reaction chamber of the nucleic acid synthesizer; because

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of the versatility of such materials, they can be readily
manipulated within the chambers, i.e. loosely "rolled" in
the case of membranes, or loosely inserted in the case of
threads. Beneficially, synthesis of oligonucleotides can

5   proceed directly onto the aminated polypropylene, and,
owing to the "activated" nature of the amine groups,
these oligonucleotides are covalently attached directly
to the polypropylene ("cleavable" links or spacer arms
can, of course, be utilized such that the

10   oligonucleotides are amenable to removal; active esters
of succinate nucleosides are preferred as these are
susceptible to "cleavage" by ammonia).

Such derived oligonucleotides are ideally

15   suited for utilization in genetic screening analyses.
I.e., by utilizing aminated polypropylene comprising
oligonucleotides, where the oligonucleotides are
complementary to either the wild-type or mutation(s)
sequence of a gene of interest, prepared patient samples

20   can be screened for the presence or absence of the
sequence of interest. It is preferred that the length of
oligonucleotides chemically synthesized for use in such
genetic analysis be up to about 250 bases in length,
preferably between about 5 and about 100 bases, more

25   preferably between about 8 and 30 bases, and most
preferably about 16 bases. These lengths are to be
construed as relative to the conditions under which the
genetic analysis is conducted. For example, at room
temperature (at which temperature we prefer to conduct

30   the analysis) the most preferred length is 16 bases; at
lower temperatures, shorter (i.e. 8-mers) can be
utilized.

The skilled artisan is readily credited with

35   understanding methodologies for preparing genomic samples
from patients for analysis. Sample DNA can be readily
obtained from, e.g., clinical samples (i.e., tears;

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semen, vaginal secretions, whole blood, serum, plasma,
skin scrapes, etc.) and readily prepared by a variety of
techniques which are available to the skilled artisan.
Typically, a primary goal of these techniques is to
5   purify the nucleic acids to a sufficient degree such that
extraneous materials which might otherwise interfere with
the eventual amplification (e.g., by the polymerase chain
reaction) of the nucleic acids of interest are removed.
Using serum as an example, preparation of the nucleic
10   acids generally can comprise the following steps:
incubate the serum for 1 hour at 70°C with proteinase k
(Boehringer Mannheim) at 2.5mg/ml in 25mM MOPS (pH 6.5),
2.5mM EDTA and 0.5% SDS. This is followed by the
following extractions: phenol extraction and ether
15   extraction. This is followed by ethanol precipitation.
See, e.g., A. Larzul, et al. J. Heptol. 5:199-204
(1987). As noted, other protocols and techniques are
readily available for such purification.

20   Following such purification it is (typically)
necessary to amplify the particular genomic region of
interest. This can be readily accomplished using
techniques such as the polymerase chain reaction ("PCR")
and primers directed to the particular region of
25   interest. The skilled artisan is credited with the
ability to appreciate and understand the application of
such amplification techniques to the genomic sample, as
well as the parameters involved therein (such as
selection of primers which will substantially ensure
30   amplification of a gene segment or region of a nucleic
acid sequence comprising a gene).

                 As will be further appreciated by the skilled
artisan, because the DNA sample, by definition,
35   comprising two complementary strands of DNA,
amplification techniques such as PCR will generate sets
of complementary amplicons (an "amplicon" is one set of

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strands of the amplification product, i.e. complementary
"amplicons" are the resulting product of a PCR
amplification). A suggested approach for increasing
sample binding to oligonucleotides covalently attached to
5   aminated polypropylene is to remove one of these sets
from the reaction mixture prior to analysis; this has the
effect of decreasing competition for hybridization of the
complementary amplicons to each other. For this
approach, only one of the amplicon sets is subjected to
10   screening with the aminated polypropylene-oligonucleotide
device. One approach to segregation of the amplicon sets
involves manipulating the primers. For example, one set
of primers can be biotinylated, and the other set can be,
e.g., labelled for detection. Thus, after amplification,
15   the biotinylated amplicons can be "removed" from the
sample using, e.g., avidin-coated beads. This has the
effect of maintaining substantially only labelled
amplicons in the solution. The label, of course, can be
utilized for detection purposes after the labelled
20   amplicons are "screened" with the aminated polypropylene-
oligonucleotide device.

Following such amplification, the sample
material can be presented to the aminated polypropylene-
25   oligonucleotide device which comprises an oligonucleotide
having a sequence complementary to the labelled
amplicons. Because a preferred application of the
aminated polypropylene-oligonucleotide devices is
screening genomic samples for genetic mutations, the
30   stringency conditions (i.e. the conditions which allow
hybridization and dehybridization to occur, including but
not limited to: temperature, ionic strength, chemical
conditions, time, and the nature of the sequences) are
important. I.e., for mutations including only one change
35   in nucleic acid sequences (vis-a-vis the wild-type, or
"normal" sequence), it is very likely that in screening
for a mutational sequence, even the normal sequence will

readily hybridize to the complementary oligonucleotide.
Thus, removal of such "non-specific" hybridization is
essential. For example, this can be accomplished using a
series of washings with decreasing salt concentrations,
which has the effect of essentially removing
substantially all of the non-specifically hybridized
materials before removing substantially all of the
specifically hybridized materials.

Because of the criticality of ensuring that
only complementary sequences are detected, it is our
current preference that an "historical" de-hybridization
analysis be conducted so that we can "track" the de-
hybridization of the sample nucleic acid sequences from
the aminated polypropylene-oligonucleotide device over
time and stringency conditions -- by comparing the de-
hybridization patterns of non-complementary and
complementary sequences as they are removed from the
aminated polypropylene-oligonucleotide device, we have
ascertained that the patterns are substantially different
such that an accurate assessment of "correct"
hybridizations can be conducted. This historical
analysis can be utilized for creating a "yes/no"
stringency protocol for specific genetic analysis. For
example, if a specific known mutation provides a specific
known "historical" de-hybridization pattern, then if this
pattern is obtained for an unknown sample, that sample
comprises that specific mutation.

In essence, we subject the hybridized material
to a decreasing salt gradient using high performance
liquid chromotography ("HPLC") techniques, while
continuously monitoring the loss of signal (indicative of
de-hybridization) over time. As will be set forth in
detail below, we have experimentally determined that
distinct differences in such de-hybridization patterns
can be ascertained, such that accurate determinations as

to the presence or absence of defined sequences can be
made. For the analysis of genomic DNA using
oligonucleotides which are synthesized directly onto,
e.g., aminated polypropylene, such analysis may be

5   readily conducted as indicated above.

       The genomic DNA typically comprises a gene or
portions thereof. As used herein, the term "gene" is
accorded the definition typically utilized by those in

10   the art. The genomic sample can be from any source
containing DNA, including, but not limited to, plants,
vertebrates, invertebrates, virus sources, etc.

       Oligonucleotides having different sequences can

15   be synthesized onto the polymer medium using various
"masking" protocols, such as those described in Maskos,
U. and Southern, E. M. "Parallel analysis of
oligodexyribonucleotide (oligonucleotide) interactions".
Nuc. Acids. Res. 20(7): 1675-1678 (1992). For example,

20   in the case of oligonucleotides, the initiation
biomonomer applied to the entire surface of a polymer
medium may be adenosine, while in only one quadrant of
the medium, the nucleotide cytidine is added, the other
three quadrants being masked, followed by the addition of

25   nucleotide thymidine to one half of the entire medium,
the other half being masked, and over the entire polymer
medium the nucleotide guanine is added. Thus, in the
first quadrant, the tetramer ACTG is present; the one
half exclusive of the first quadrant includes the trimer

30   ATG; and the other half includes the dimer AG. Such a
material can be utilized for genomic analysis or for
screening for genetic mutations which include various
alleles. For example, there are at least 95 different
nucleic acid sequences that can give rise to the gene

35   which encodes for the disease cystic fibrosis. Thus, a
single polymer device medium can be generated with 95
different biopolymers having different sequences thereon,

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each of the biopolymers being generated in accordance
with a masking protocol as described above.

          Alternately, one can synthesize different
biopolymers onto different polymer mediums and combine
these onto a single polymer device. For example, in the
case of membranes, various sections from different
polymer media comprising different oligonucleotides can
be affixed to a single device. The benefit of this
approach is that one can more readily quality control a
portion of the polymer medium comprising the biopolymer
such that the quality of a device comprising a portion of
that polymer medium can be assured. In the case of
cystic fibrosis, e.g. 95 separate polymer media (or a
sub-population thereof) comprising 95 different
oligonucleotides can be utilized, with each being
dissected and portions from each being subjected to
quality control procedures. Thereafter, these 95 media
can be combined onto one (or several) unitary device
medium.

          The disclosed polymers can also be utilized for
the parallel analysis of oligonucleotides, or "Sequencing
by Hybridization" ("SBH"). One of the earliest and
practical approaches to SBH is disclosed by Edwin M.
Southern in PCT Publication No. WO 89-10977, "Analyzing
Polynucleotide Sequences". See also, Maskos & Southern,
supra (hereinafter "Southern SBH"). According to the
Southern SBH protocol, many different oligonucleotides
are synthesized onto the surface of a solid support and
then utilized to carry out parallel hybridizations with
sample nucleic acid sequences; the oligonucleotides are
synthesized in situ which ensures uniformity of yield of
the oligonucleotides. Beneficially, carrying out
hybridization to all the different sequences on the same
surface in a single analytical run results in identical
experimental conditions for every individual

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oligonucleotide. Surface activated polymer medium as
disclosed herein is directly applicable to SBH protocols.
Surface activated polymer media are also applicable to
the preparation of polymers utilizing photoremovable
5   protecting groups. See, PCT Publication No. WO 90/15070,
"Very large scale immobilized peptide synthesis".

        The disclosed surface activated polymers are
particularly suited for in situ biopolymer synthesis for
10   "reverse dot blot" protocols. For example, in certain of
the protocols described above, a series of
oligonucleotides having different defined sequences are
synthesized directly onto a surface activated polymer (or
synthesized "off-line" and attached to the polymer).
15   Thereafter, a sample suspected of containing a
polynucleotide having a sequence complementary to one of
the oligonucleotides is applied thereto. Detection can
be accomplished via a labelling scheme that provides for
labelling of the polynucleotide prior to analysis (direct
20   labelling) or after hybridization (indirect labelling).
Because the investigator knows the physical location of
the different oligonucleotides on the polymer medium, the
presence of a label at a specific location provides
information as to both the presence and the sequence of
25   any material from the sample that has hybridized to the
aminated polypropylene oligonucleotide device.

        Additionally, the disclosed surface activated
polymer materials can also be utilized in conjunction
30   with linkers and spacer arms for the synthesis of
biopolymers. These can be amenable to either non-
cleavage or cleavage protocols for non-removal or removal
of the synthesized biopolymer from the polymer support.
Linkers and spacer arms applicable to the synthesis of
35   biopolymers, particularly oligonucleotides and peptides,
are well known and varied and will not be discussed
herein in detail. Thus, the disclosed polymer materials

can be used for the synthesis of, e.g., oligonucleotides or peptides which can in turn be cleaved from the support and utilized in any of a variety of contexts as set forth above.

The disclosed surface activated, organic polymers are also applicable to protein/peptide sequencing. As those in the art appreciate, protein sequencing, unlike protein synthesis. is directed to the determination of the amino acid sequence of a particular protein; the amino acid sequence of a particular protein can then, in turn, be utilized for, e.g., chemical synthesis of that protein or determination of a general or optimal nucleic acid sequence of codons which will encode for the amino acids of the protein. A well known procedure for protein sequencing is referred to as "Edman degradation." See, Edman, P. and Henschen, A. (1975) in: Protein Sequencing Determination (Needleman, S.B. ed.) pp. 232-279. Springer-Verlag, Berlin, Heidelberg, New York. Briefly, the Edman degradation process involves the cleavage of amino acid residues, one at a time, from the N-terminus of the peptide, and identification thereof as phenylthiohydantoin derivatives. This procedure is well characterized and will not be set forth in detail herein. Automated protein and peptide sequencers are commercially available; exemplary is the Porton LF 3000 Protein Sequencer (Beckman Instruments, Inc.)
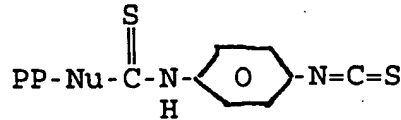
Beneficially, a protein to be sequenced can be covalently attached to a surface activated, chemically inert organic polymer using, e.g., 1,4 phenylene diisothiocyanate ("PDITC"); however, any moiety capable of binding to the surface of the polymer and a protein can be utilized. The coupling reaction can proceed as follows:

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$$PP\text{-}Nu \quad + \quad S=C=N-\hspace{-2mm}\langle\!\!\langle\; O\;\rangle\!\!\rangle\hspace{-2mm}-N=C=S \quad (\text{"PDITC"})$$

↓

$$PP\text{-}Nu\overset{\displaystyle S}{\overset{\|}{-C}}\overset{}{-N}-\hspace{-2mm}\langle\!\!\langle\; O\;\rangle\!\!\rangle\hspace{-2mm}-N=C=S$$
$$\phantom{PP\text{-}Nu-C-}\underset{H}{}$$

↓ Protein, pH 8-10

$$PP\text{-}Nu\overset{S}{\overset{\|}{-C}}\underset{H}{-N}-\hspace{-2mm}\langle\!\!\langle\; O\;\rangle\!\!\rangle\hspace{-2mm}\overset{S}{\underset{H}{-N}}\overset{\|}{\underset{}{-C}}\underset{H}{-N}-\text{Protein}$$
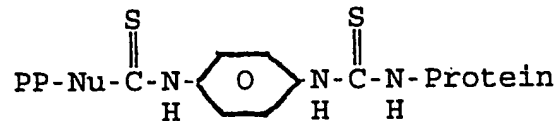
where: "PP-Nu" is polypropylene comprising a nucleophile
adsorbed on the surface thereof.

The protein, being covalently attached to the polymer,
can then be efficiently sequenced using, e.g., Edman
degradation protocols.

Another application for the disclosed surface
activated, chemically inert organic polymers can be
directed to the covalent attachment of biomonomers such
as avidin, avidin derivatives, biotin, and biotin
derivatives. Such materials have a plethora of
applications -- particularly preferred is sequestering
biotinylated or avidin-linked macromolecules,
respectively. As those in the art appreciate, avidin is
a glycoprotein having four binding sites specific for
biotin; the binding affinity between biotin and avidin is
both very strong and non-covalent. The utility of
avidin, for example, attached to such a polymer is varied
-- as an example, such a material can be utilized to
standardize the resulting sequence reaction of the
amplification of a polynucleotide containing sample
using, e.g., the PCR. For example, biotinylated primers
(specific to a first region of a target sequence) are
added to a polymer device medium having avidin covalently
coupled thereto; thereafter, second, labelled primers
(specific to a second region of the target), polymerase

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enzyme, and nucleotide triphosphates are added to the
bound primer and the PCR reaction is initiated. By
controlling the amount of avidin attached to the polymer,
the amount of biotinylated primer is "controlled", such

5   that elongated, labelled strands which result form the
PCR reaction are standardized, and these can then be
analyzed utilizing, e.g., conventional slab gel
electrophoresis techniques.

10   The surface activated, organic polymers can
also be utilized for cell adhesion and cell
growth/propagation for example, in devices used for
mammalian cell culture, artificial skin grafts and
prosthetic devices exhibiting tissue and blood

15   compatability. For example, bioreactive peptides
directed to specific cell recognition domains can be
synthesized onto the disclosed polymers, and samples
comprising a variety of cells can be applied thereto,
whereby those cells comprising the recognition domains

20   can selectively attach thereto. Yamada, K.M., "Adhesion
Recognition Peptides," J.Bio.Chem. 266:20 12809-12812
(1991), describes a variety of bioreactive peptides and
the cells comprising recognition domains specific for
such domains. For example, the adhesive glycoprotein

25   fibronectin is involved in a variety of biological
processes, particularly in mediating cell attachment and
cell migration. Fibronectin is bound by several cell
surface receptors; peptide sequences of fibronectin which
are recognized by such receptors include Arg-Gly-Asp

30   ("RGD") and Leu-Asp-Val ("LDV"). Thus, a series of
biopolymers comprising one or more RGD peptides can be
synthesized onto the disclosed polymers to, e.g., mediate
cell attachment thereto. For example, Pierschbacker and
Ruoslahti, Nature 309:30-33, 1984 (and cited references),

35   first isolated and characterized a peptic digest fragment
of fibronectin containing the cell attachment domain. A
30-amino acid synthetic peptide was prepared which

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carried the cell attachment promoting activity. The
domain was further delineated to a tetrapeptide (RGDS)
that promoted the attachment of rat kidney fibroblasts
when attached via a 6-carbon atom spacer arm to Sepharose
5   beads but not when the RGDS tetrapeptide was coupled to
protein-coated plastic plates. The authors suggested
that this lack of activity might be due to a decrease in
accessibility of cells to the attachment domain or
because of poor coupling efficiency to the protein-coated
10   plastic surface. To alleviate this problem, Cappello and
Crissman, Polymer Preprints 31: 193-194, 1990, utilizing
recombinant genetics, inserted a 10-amino acid sequence
of fibronectin that contained the RGD domain into a
segment of the amino acid sequence encoding the
15   crystalline region of the Bombyx mori silk fibroin
protein. A high molecular weight copolymer SLP-F
containing repeat sequences of the RGD recombinant
peptide was immobilized onto nitrocellulose filters and
promoted the attachment of african green monkey kidney
20   epithelial cells. The supports disclosed herein can be
utilized in an efficient manner to achieve these types of
end-results.

Preferably, bioreactive peptides are
25   synthesized using a spacer arm to spatially distance the
bioreactive peptides from the surface of the polymer.
Such spacer arms are preferably about 50 atoms in length,
more preferably about 20 atoms in length, and most
preferably about 6 atoms. A preferred spacer arm is
30   hydrophilic. Preferably, the bioreactive peptide
sequence is repeated along a biopolymer comprising other
non-bioreactive peptide sequences; this is preferred
because an increase in the number of bioreactive peptide
sequences increases the probability that the recognition
35   domains of a cell will bind to the bioreactive peptide.
Preferably, the bioreactive peptide sequence is repeated
at least about 30 times within a biopolymer, more

preferably about 15 times along the biopolymer, and most
preferably about 10 times. It is preferred that the
distance between any two bioreactive peptide sequences be
at least about 1 amino acid residue, more preferably
5   about 3 and most preferably about 9.

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EXAMPLES

The following examples, which are neither
5   intended nor to be construed as limiting, are directed to
a particularly preferred embodiment of the invention --
the amination of polypropylene, followed by the direct
synthesis of oligonucleotides thereon, for use in the
analysis of samples comprising nucleic acid sequences,
10   for an indication of the presence of non-conforming
sequences, i.e. mutations; and the direct synthesis of
peptides thereon.

I.   MATERIALS, METHODS, INSTRUMENTS AND SPECIFIED
15   OLIGONUCLEOTIDES

A.   Reagents

1)   OLIGO 1000 DNA Synthesizer
20   Synthesis of oligonucleotides was accomplished
using Beckman Instruments, Inc. BINARY-PAK™
phosphoramidites (dA(Bz) - Part No. 337737, dC(Bz) - Part
No. 337738, dG(iBu) - Part No. 337739, T- Part No.
337746); DNA Synthesis Reagent Kit (Oxidize - Part No.
25   337732, DEblock - Part No. 337733, Cap 1 - Part No.
337734, Cap 2 - Part No. 337735); ACtivate Reagent (Part
No. 338284) and Cleavage and Deprotection Kit (Part No.
337742).

30   2)   Hybridization Buffer
All chemicals were at least of ACS grade.
Hybridization buffer consisted of 6XSSPE/0.01% sodium
dodecyl sulfate. 6XSSPE was prepared by dilution of
20XSSPE (per liter: 3M NaCl; 0.2M NaH_2PO_4; 0.02M EDTA;
35   adjusted to pH 7.4 with 10N NaOH) to achieve 6X, followed
by addition of 0.01% SDS (v/v) thereto.

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    3)   D-HAS™ Gradient Buffer
        D-HAS™ gradient buffer consisted of
2XSSPE/0.01% SDS (i.e., utilizing 20XSSPE stock, diluting
to 2XSSPE, and addition of 0.01% SDS (v/v).)

    4)   Radiofrequency Plasma Reagents
        Electronic grade anhydrous ammonia (99.995%
purity) was obtained from Liquid Air Corporation (Walnut
Creek, CA) under the brand name ALPHAGAZ™. Ultra high
purity argon gas (99.999%) was also obtained from Liquid
Air under the same brand name.

B.   Polymer Device Media

        Polypropylene membrane filter sheets (21.5cm x
26.6cm, 0.2μm pore size) were obtained from Gelman
Sciences Ann Arbor, Michigan under the brand name
METRICEL™. Polypropylene threads (0.010 inch diameter)
were obtained from Blue Mountain Industries, Blue
Mountain, Alabama (Product No. MP69). Polypropylene film
(1.2 mil) was obtained from Catalina Plastics and Coating
Corporation (Calabasas, CA.).

C.   Commercially Available Protocols

    1)   Polymerase Chain Reaction ("PCR")
        Amplification of specified DNA macromolecules
was accomplished using PCR protocols using a Perkin-Elmer
Cetus GeneAmp™ DNA Amplification Reagent Kit with
AmpliTaq™ (Part No. N801-0055). Manufacturer
instructions were followed.

    2)   Primer Biotinylation
        5'-biotinylated primers for PCR amplification
were made using Biotin-ON™ phosphoramidite (Clonetech
Laboratories, Inc., Palo Alto, Ca. Part No. 5191).

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Biotinylated primers were not purified prior to
utilization. Manufacturer instructions were followed.

3) Primer Labelling
Primers for PCR amplification were 5'-labelled
with γP^{32} (Amersham, United Kingdom) using USB Cloned T4
Polynucleotide Kinase reagents. Manufacturer
instructions were followed.

4) Probe Labelling
615 fluorescein probe was labelled with
Fluorescein-ON phosphoramidite at the 5' end (Clonetech,
Cat. No. 5235-1). Manufacturer instructions were
followed.

5) Sulfo-SDTB Analyses - Amine Content,
Quantitative
Amine content on polypropylene device media was
determined, inter alia, using the Sulfo-SDTB Assay
(Pierre Chemical; Product No. 28610X). Quantitation was
achieved by measuring the release of DMT cation from the
device media under acidic aqueous conditions. The amount
of DMT generated was estimated from the extinction
coefficient at 498 nm of 70,000 $cm^{-1} M^{-1}$ or from standard
curves (ΔOD 498 nm vs. Sulfo-STDB standards).
Manufacturer instructions were followed with the
exception that 6N HCl was used to release DMT cation into
solution.

D. Additional Analytical Protocols

1) Ninhydrin Analysis - Amine Content, Qualitative
Qualitative analysis of aminated polypropylene
was conducted by ninhydrin analysis; ninhydrin, a
heterocyclic compound, complexes with amine groups and
during the complexation process, a color change, from
yellow to a deep blue, occurs. Approximately 1-3 drops

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of each of the following solutions were added serially to putative aminated polypropylene material: A - potassium cyanide/pyridine (0.01M KCN/98ml pyridine); B - 500 mg ninhydrin/10ml butanol; C - 80mg phenol/20ml butanol. This was followed by heating of the materials at 110°C for about 2min, followed by qualitative observation of color.

        2)   DMT Analysis - Amine content, Quantitative
        Quantitative determination of amination of polypropylene was accomplished using a modification of the procedure set forth in Reddy, M.P. et al. "An efficient procedure for the solid phase tritylation of nucleosides and nucleotides." Tetrahedron Letters 28/1: 23-26 (1987). Amine groups react with dimethoxytrityl (DMT) chloride in the presence of tetra-n-butylammonium perchlorate/2,4, 6-collidine in dichloromethane; upon acid treatment, DMT cation is released and can be measured spectrophotometically at 501 nm. Results can be presented as OD units/cm^2 of aminated polypropylene.

        The protocol was as follows: aminated polypropylene was suspended in equimolar solution (0.5M) of dimethoxytrityl chloride (Aldrich, St. Louis, MO) and tetra-n-butylammonium perchlorate (Fluka, City, State) in dry dichloromethane containing 2,4,6-collidine. The reaction was completed within between about 15 to 30 minutes. The aminated polypropylene was removed and washed thoroughly with dichloromethane. Dry aminated polypropylene was thereafter suspended in 10ml of 2% trichloroacetatic acid in dichloromethane (w/v); the presence of an orange color indicated the presence of DMT cation, and this was quantified spectrophotometrically (λmax - 501 nm, ϵ = 76,000).

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E.   Instruments

1)   Radio Frequency Gas Glow Plasma Discharge

A Plasma Sciences Instrument Model 0150E was utilized to generate a radiofrequency gas glow discharge in the presence of ammonia gas.

The amination process consisted of the following steps: a base pressure of 0.1 torr was established within the chamber at the initiation of, and between each, amination process, under a continuous vacuum sweep operation. Thereafter, anhydrous ammonia was introduced for 4min to obtain a chamber pressure of about 0.25 torr. This was followed by application of RF-power at 200 watts for 2min in the presence of the anhydrous ammonia gas. Following discontinuation of RF-power, the chamber was maintained in ammonia gas for an additional two minutes at the same pressure. This was followed by the introduction of argon gas into the chamber for 10min at about 0.25 torr. Finally, the chamber was returned to atmospheric conditions under a slow vent to on for about 1min.

2)   Automated DNA Synthesizer

Synthesis of oligonucleotides was performed on a Beckman Instruments, Inc. (Fullerton, CA) OLIGO 1000 automated DNA synthesizer using phosphoramidite-based chemistry protocol. Aminated polypropylene was utilized for the solid support material. Homo- and hetero-oligonucleotides of various lengths were synthesized in accordance with manufacturer instructions.

3)   Capillary Gel Electrophoresis ("CGE")

Capillary electrophoresis analysis of oligonucleotides was performed on a Beckman Instruments, Inc. P/ACE™ 2000 high performance capillary

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electrophoresis system. A 27cm, 100μm i.d., column
(Polymicro Technologies, Inc., Phoenix, AZ) was utilized;
inpolymerized polyacrylamide gel column was prepared in-
house using 10%T. Samples were loaded onto the columns
via the electrokinetic injection method (7.5kV; 3.0
sec.); separation was conducted at 300V/cm for 10-30 min,
depending on oligonucleotide length. Tris-hydroxymethyl
amino methane ("TRIS")-borate (pH 8.3) was utilized as
the running buffer. Absorbance detection was in the
range of from 0.01 to 1.0 OD_{260nm}/ml, depending principally
on the length of the oligonucleotide.

    4)   High Pressure Liquid Chromatography ("HPLC")
          HPLC analysis of oligonucleotides was conducted
on a Beckman Instruments, Inc. System Gold™ HPLC
Programmable Solvent Module 126 equipped with a diode
array detector module 168 and autosampler 507 (20μl
injector loop). C18 Ultrasphere™ HPLC column (Beckman,
Part No. 244254; 3μ particle ODS, 4.6mm x 7.5cm) was
utilized. Bottle A contained 0.1M ammonium acetate, pH
6.8; Bottle B contained HPLC-grade acetonitrile. The
system was operated in a gradient mode as follows (flow
rate = 1ml/min): 0-3min - 100% Bottle A, 0% Bottle B; 3-
33min - 100% Bottle A to 30% Bottle A/0% Bottle B to 70%
Bottle B.

    5)   Breadboard Dynamic Hybridization Analysis
          (D-HAS™) System
          In order to analyze the progressions of probe-
target disassociation over time, a breadboard Dynamic
Hybridization Analysis (D-HAS™) System was constructed.
For the D-HAS™ analyzer used herein, a Beckman
Instruments, Inc. System Gold™ HPLC Programmable Solvent
Module 126 equipped with a modified 171 radioisotope
detector was utilized; the modification consisted of
replacing the flow cell with a 1/8 inch o.d./ 1/16 inch
i.d. fluoronated ethylene propylene copolymer tubing.

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This tubing allowed for insertion of aminated polypropylene-oligonucleotides having labelled sequences of interest hybridized thereto therein, and this material was in turn "sandwiched" between 2 polypropylene screens.
5   This arrangement allowed for the flow of Disassociation Buffer through the modified flow cell -- thus, as disassociation of the labelled sequence from the aminated polypropylene oligonucleotide occurred, the number of radioactive counts decreased, thus providing a continuous
10 tracking of the disassociation of the probe from the target. Bottle A contained D-HAS™ gradient buffer and Bottle B contained 0.01% SDS. The D-HAS™ System was operated in a gradient mode as follows: 0-2min - 100% Bottle A (1ml/min); 2-22min - 0% -100% Bottle B
15 (2ml/min); 22-24 min - 100% Bottle B (2ml/min); 24-26min - 0% - 100% A (2ml/min).

        6)   Charged Coupled Device ("CCD") Camera
               For detection of fluorescently-labelled probe,
20 a Photometrics Metachrome 2 CCD Array camera (Tuscon, AZ.) in conjunction with Photometrics Nu200 Camera Controlled Software Rev. 2.0. was utilized. Laser source was an argon ion laser, 457-514nM (OmniChrome, Chino, CA.).
25

        D.   Oligonucleotide Sequences
               Oligonucleotides utilized throughout the Examples had the following sequences (for ease of presentation, the Examples will refer to the oligonucleotides by the
30 listed identifiers):

35

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1. **TARGET A (SEQ ID NO. 1)**:

    3'-CCA CAT TTC GGT TGT G-5'

5

    The 3' end of Target A was directly synthesized to
    the aminated polypropylene (i.e. no "linker" was
    utilized).

10 2. **P23 (SEQ ID NO. 2)**:

    5'-GGT GTA AAG CCA ACA C-3'

    P23 is a labelled complement to Target A (SEQ ID No.

15 1)

    3. **P24 (SEQ ID NO. 3)**:

    5'-GGT GTA A<u>G</u>G CCA ACA-3'

20

    P24 is a labelled "complement" probe to Target A
    with the exception of the underlined base, G (this
    base should be an A).

25 4. **P37 (SEQ. ID NO. 4)**:

    5'-GGT GTA AA..CA ACA C-3'

    P37 is a labelled "complement" probe to Target A
30 with the exception of a two base deletion ("..")
    which should be "GG".

    5. **TARGET A61 (SEQ ID NO. 5)**:

35 . 3'-T TTA TAG TAG AAA CCA-5'

The 3' end of Target A61 was directly synthesized to
the aminated polypropyelen.

6.   TARGET A70 (SEQ ID NO. 6):

5

3'-T TCT TTT ATA GTA GAA-5'

The 3' end of Target A70 was directly synthesized to
the aminated polypropylene.

10

7.   CFTR Exon 10, Normal (SEQ ID NO. 7):

5'-G TTT TCC TGG ATT ATG CCT GGC ACC ATT

AAA GAA AAT ATC ATC TTT GGT GTT TCC

15   TAT GAT GAA TAT AGA TAC AGA AGC GTC

ATC AAA GCA TGC CAA C-3'

8.   CFTR Exon 10, ΔF508 (SEQ ID NO. 8):

20   5'-G TTT TCC TGG ATT ATG CCT GGC ACC ATT

AAA GAA AAT ATC ATT GGT GTT TCC TAT

GAT GAA TAT AGA TAC AGA AGC GTC ATC

AAA GCA TGC CAA C-3'

25   9.   CFTR Exon 10 PCR Primer, Sense (SEQ. ID. NO. 9):

5'-G TTT TCC TGG ATT ATG CCT GGC AC-3'

This primer was biotinylated at the 5' end.

30

10. CFTR Exon 10 PCR Primer, Antisense (SEQ ID NO. 10):

5'-G TTG GCA TGC TTT GAT GAC GCT TC-3'

35   This primer was labelled at the 5' end.

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11. <u>Cleavable Target 17-mer/Qualitative (SEQ. ID. NO. 11)</u>:

3'-TCA GCT ACC GTA AAT GT-5'

12. <u>SAM 125 (SEQ. ID. NO. 14)</u>:

3'-AAG GAC CTA ATA CGG-5'

The 3' end was directly synthesized to aminated polypropylene film.

13. <u>615 Fluorescent Probe (SEQ. ID. NO. 15)</u>:

5'-GTT <u>TTC CTG GAT TAT GCC</u> TGG GAC

The 5' end of the probe was labelled with the fluorescein label; the underlined portion of the probe is complementary to SAM 125

EXAMPLE I
Amination of Polymer Device Media

5   Polypropylene membrane filter sheets and films
were subjected to the RF amination procedure as
delineated above. Following plasma amination,
approximately 1cm x 1cm edge cuts of the sheets and films
were qualitatively analyzed for amine content by the
10   ninhydrin reaction: non-aminated ("virgin")
polypropylene control sheets and films evidenced no color
change (i.e. the color remained yellow); aminated
polypropylene sheets evidenced a blue color, indicative
of amine groups present on the polypropylene sheets. The
15   remainder of the sheets and films were stored at room
temperature (in dark) in polyethylene bags, heat sealed
using an electric wire impulse heat sealer. Prior to
oligonucleotide synthesis, sheets and films were
quantitatively analyzed for amine content by Sulfo-SDTB:
20   virgin polypropylene evidenced about 0 to 1 nmoles/cm^2;
polypropylene membrane subject to RF treatment was
determined to comprise between about 5 to 30 nmoles/cm^2.

          Polypropylene threads were subject to the RF
25   amination procedure as delineated above, with two
exceptions: substantially linear threads were placed
upon the glass plates and held in-place at the ends
thereof using an adhesive tape, or threads were coiled
around a cylinder core of polypropylene open mesh
30   material, followed by attachment of the cores (via the
ends thereof) to the glass plates using an adhesive tape.
Approximate lengths of 6-18cm of threads were utilized
for determination of amination. For qualitative
analysis, virgin polypropylene thread did not evidence a
35   blue color when subjected to ninhydrin testing protocols,
while polypropylene threads subjected to the RF treatment
evidenced the desired blue color. Quantitative testing

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(via Sulfo-SDTB) indicated that virgin polypropylene
threads had an amine surface content of between about 0
to 2 nmoles/cm^2; polypropylene threads subjected to RF
treatment evidenced an amine content of between about 5
5   to 30 nmoles/cm^2.

       Masking of polypropylene sheets (21.5cm x
26.6cm) was accomplished by placing such sheets on the
glass plate within the RF instrument and overlaying the
10   sheet with a 30cm x 30cm polypropylene mesh filter screen
(Spectra/Mesh Los Angeles, CA, Prod. No. 146410, 1000 μm
x 1000 μm nominal mesh opening). Plasma amination was
conducted as delineated above. Qualitative testing for
amine "patterning" (i.e. the presence of amine groups
15   corresponding to the nominal mesh openings) was conducted
using Sulfo-SDTB reagent, followed by rinsing in
distilled water, followed by holding the sheets over
fumes of concentrated hydrochloric acid. A "checkered"
pattern resulted where the unmasked areas had an orange
20   color (indicative of the presence of amine groups) while
the areas beneath the mesh portion (masked area) was
white (indicative of the absence of amine groups). The
same sheet was then rinsed in distilled water, methanol
and acetone, followed by air drying. Thereafter, a 30
25   dyne-cm blue dye wetting Tension Test System Kit No. 5
(Select Industrial Systems, Waukesha, WI) was applied to
the polypropylene sheet; the area covered by the mesh
adsorbed blue dye, indicating that this area was
hydrophobic (i.e. amine groups absent), while the
30   unmasked areas remained white in appearance, indicating
that this area was hydrophilic (i.e. amine groups
present).

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EXAMPLE II
Determination of Oligonucleotide Presence
on Aminated Polypropylene

Determination of the efficiency of
oligonucleotide synthesis onto aminated polypropylene of
Example I was predicated upon HPLC and CZE analysis of
oligonucleotides synthesized onto the material and
cleaved therefrom.

It is important that the support material allow
for substantially identical synthesis on the surface
thereof -- i.e., if the support material allows for
variation in synthesis, oligonucleotides having different
lengths may be synthesized thereon.

An efficient means for determining the quality
of the synthesized material is by HPLC and/or CZE
analysis of the synthesized material. If the support
material allows for consistent and efficient synthesis,
then the material that was synthesized thereon, removed,
and subjected to HPLC and/or CGE analysis should be
expected to generate a single peak. I.e., there are no,
or substantially minor, "contaminants", present, these
being indicative of oligonucleotides not having the
correct sequence length and composition.

Prior to oligonucleotide synthesis, and owing
to the objective of analyzing the synthesized material, a
linker group was added to the aminated polypropylene.
Specifically, the aminated polypropylene was condensed
with the active ester of nucleoside succinate; this was
followed by the addition of other nucleosides. As is
appreciate, the succinate portion is amenable to
"cleavage" using ammonia.

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17-mer oligonucleotides (SEQ ID NO. 11) were
synthesized directly onto aminated polypropylene sheets
(.5cm x 1.5cm) which were hand-rolled and loosely packed
into a needle-tip reaction column of a Beckman
5   Instruments OLIGO 1000 DNA Synthesizer; aminated
polypropylene threads (about 20cm in length) were
similarly utilized. Following synthesis of the 17-mer
oligonucleotides, the oligonucleotides were cleaved from
the supports with NH_4OH(28%) for 1hr. at room temperature,
10   followed by deprotection with NH_4OH(28%) for 1hr. at 80°C.
Released oligonucleotides were then analyzed by HPLC and
CGE techniques under the parameters set forth above.
Results were presented in Fig. 1 (HPLC) and Fig. 2 (CGE)
for oligonucleotides synthesized onto polypropylene
15   sheets (results for polypropylene threads yielded
substantially identical results; these results are not
provided herein).

As is evident from Figs. 1 and 2, a single,
20   well defined peak is set forth, indicating, inter alia,
that synthesis efficiency on the aminated polypropylene
was optimal (the small peak in Fig. 1 is attributed to
benzamide formed by the removal of benzoyl protecting
groups on the synthetic DNA strand).

25

30

35

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EXAMPLE III
Dehybridization Chracteristics of
Complements and Mutations

In order to determine the hybridization
characteristics of polynucleotides to target sequences
synthesized onto aminated polypropylene, a series of
experiments were performed using oligonucleotides which
had sequences which were perfect complements (i.e.
analogous to "wild-type") and sequences which were not
perfect complements (i.e. analogous to "mutations"). As
noted, in genetic analysis, it is critical to
differentiate between wild-type and mutation sequence;
this goal is exacerbated given the ability of deleterious
mutations to be considered by a single base
deletion/substitution and the preonderance of such a
mutated sequence to hybridize to a wild-type complement
probe. Because of these factors, dehybridization from
the target was analyzed over time using the D-HAS™
System.

Previously referenced Target A was directly
synthesized onto aminated polypropylene membranes using
the aforementioned DNA synthesizer. Thereafter, P23
(perfect compliment to Target A); P24 (single base
mismatch); and P37 (two-base deletion) were separately
introduced to Target A as follows: aminated
polypropylene having Target A covalently bound thereto
was equilibrated in Hybridization Buffer; thereafter,
each of the three probes (0.5pmoles/50µl Hybridization
Buffer) were added to these membranes followed by 1hour
incubation at 25°C. Hybridization Buffer was then
removed, and membranes rinsed once with 200µl
Hybridization Buffer; thereafter, membranes were added to
the D-HAS™ System as disclosed. Analysis of decreasing

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presence of labelled probes was conducted, with results
being collectively presented in Fig. 3.

5   It would be expected that between a "perfect"
complement to Target A and a "non-perfect" complement,
the perfect complement should remain hybridized to Target
A longer than the non-perfect complement under identical
conditions. Fig. 3 evidences the validity of this
expectation. It is noted that the difference in
10   dehybridization patterns between P23 (the complement) and
P24 (single base mismatch) is quite evident; the
difference in dehybridization patterns between P23 and
P37 (two-base deletion) is even more striking.

15   These results indicate, inter alia, that wild-
type target and mutation target(s) can be synthesized
directly to aminated polypropylene and these can be used
for genetic screening -- the ability to differentiate
between the presence of wild-type complement and mutation
20   (or vice-versa) is evident.

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EXAMPLE IV
Analysis of Cystic Fibrosis ΔF508 Exon 10

5   Amplicons derived from CRTR Exon 10, ΔF508
patient sample (SEQ ID NO. 8) and CFTR Exon 10, normal
patient sample (SEQ ID NO. 7) were provided by Professor
C. Thomas Caskey (Baylor Medical College, Houston, TX).
These were amplified using the sense and antisense
10   primers described; following amplification, biotinylated
amplicons were removed (using avidin coated beads) such
that substantially only labelled amplicons were utilized
for analysis.

15   Focusing on CFTR Exon 10, the underlined
portion of that Sequence (SEQ ID NO. 7) is referred to
herein as the "regional mutation," i.e. in ΔF508, the
bases CTT are deleted such that ATC TTT is presented as
ATT. We have determined that in constructing a target
20   for the regional mutation to be synthesized onto the
surface activated organic polymer, it is preferred that
the complement to the regional mutation along the target
should be located so as to maximize the number of
possible mis-matches when the mutation is present. For
25   example, if the regional mutation is located along the
target distal to the aminated polypropylene, then the
corresponding hybridizations of, in this example, Exon
10,ΔF508 and Exon 10,Normal, are as follows: (the
underlined portion is the complement to the regional
30   mutation):

          3' TTC T TTT ATA GTA GAA 5'   Target A70
          5' AAG A AAA TAT CAT CTT 3'   Exon 10, Normal
          5' AAG A AAA TAT CAT [TGG] 3'   Exon 10, ΔF508

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Thus, focusing on Exon 10,ΔF508, along the 16-mer Target
A70, when Exon 10,ΔF508 hybridizes thereto, there will be

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13 complementary bases, and 3 mis-matches (indicated in brackets). By shifting the complement to the regional mutation toward the polymer, the number of mis-matches increases:

      3' TTTATAGTA_GAA_ACCA - 5'   Target A61
      5' AAATAACATCTTTGGT - 3'   Exon 10, Normal
      5' AAATAACAT[TGGTGT]T - 3'   Exon 10, ΔF508

This shift decreases the number of complementary bases on a Target A61-Exon 10,ΔF508 hybridization to 10, and increases the number of mis-matches to 6, i.e. a 100% increase in mis-matches.

The intent is that the number of mis-matches should be maximized. This will, of course, depend upon the length of the regional mutation. However, a preferred percentage of mis-matches between a wild-type complement target and a sample mutation is at least about 20%, more preferably about 40%, and most preferably less than about 50% (if the number of mis-matches bases exceeds about 50%, stringency conditions may not allow for sufficient hybridization of a mutation to the target). However, these percentages are relative to the position of the mismatch on the target and the type of mismatch. For example, distal mismatches are less preferred than internal mismatches, and the G-C content involved in the complementary hybridization are typically "stronger" than A-T content.

For the analysis of the Exon 10,ΔF508 and Exon 10,Normal amplicons, Target A61 was utilized; Target A61 was synthesized directly onto aminated polypropylene as described above. Hybridization and dehybridization conditions were as set forth in Example III. Results are presented in Fig. 4.

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        ·As the results of Fig. 4 indicate, the
difference in dehybridization between the CFTR Exon 10,
Normal and. CFTR Exon 10,ΔF508 from the wild-type
complement Target A61 is striking -- the pattern for the
5   dehybridization of Exon 10, ΔF508 is nearly identical to
that for background noise. Thus, analysis of samples for
the presence of genetic mutations can be readily
accomplished.

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EXAMPLE V

Analysis of Hen-Egg Lysozyme ("HEL") Peptides

5   The following peptides were synthesized
directly onto separate aminated polypropylene membranes.
These hen-egg lysozyme peptides had the following
sequences:

10   HEL 11-25 (SEQ. ID. NO. 12):

                Ala-Met-Lys-His-Gly-Leu-Asp-
                Asn-Tyr-Arg-Gly-Tyr-Ser-Leu

15   HEL 106-116 (SEQ. ID. NO. 13):

                Asn-Ala-Trp-Val-Ala-
                Trp-Arg-Asn-Arg-Cys-
                Lys

20

The carboxyl-group of Leu (HEL 11-25) and Lys (HEL 106-
116) were coupled directly to the aminated polypropylene
(i.e. Leu and Lys, respectively, were the initiator
biomonomers). The peptide synthesis was conducted in
25   accordance with the general protocol described in D.
Hudson; J. Org. Chem. 53:617 (1988); Milligen Technical
Note 4-30, (1987). Fmoc-protected amino acids were
obtained from Beckman Instruments, Inc. (Prod. Nos. Fmoc
Cys (Trt): 266366; Fmoc Lys (TBoc): 266387; Fmoc Asn:
30   266351; Fmoc Trp: 266408; Fmoc Ala: 266342; Fmoc Val:
266414; Fmoc Leu: 266384; Fmoc Ser (OTBu): 266402; Fmoc
Tyr (OTBu): 266410; Fmoc Gly: 266375; Fmoc Asp (OTBu):
266354; Fmoc His (Trt): 266377; Fmoc Met: 266390; Fmoc
Arg (Mtr) was obtained from Milligen (Prod. No. 911014).
35   Coupling reagent (1,3-di-isopropyl carbodiimide) was
obtained from Aldrich (Prod. No. D12, 540-7). Coupling
agent (hydroxybenzotriazole) was obtained from Aldrich

-61-

(Prod. No. 15,726-0). Fmoc-deblocking group (piperidine) was obtained from Aldrich (Prod. No. 10,409-4). Side-chain groups were removed using 19ml trifluoro acetic acid (95%) (Aldrich, Prod. No. 29,953-7), 0.5ml anisole

5   (Aldrich, Prod. No. 12,322-6) and 0.5ml ethyl methyl sulfide (Aldrich, Prod. No. 23,831-7), which were added to the mixture and left at room temperature for six hours, followed by washing with ether.

10   To validate the presence of these particular peptides, murine anti-HEL-11-25 monoclonal antibody and murine anti HEL-106-116 monoclonal antibody were utilized in an ELISA format. Antibodies were graciously provided by Dr. Clifford Olson, Beckman Instruments, Inc. These

15   antibodies do not cross-react with these peptides. Three conditions were analyzed: A - aminated polypropylene membrane comprising the HEL-11-25 peptide directly synthesized thereon; B - aminated polypropylene membrane comprising the HEL-106-116 peptide directly synthesized

20   thereon; C - control (aminated polypropylene membrane). ELISA conditions were as follows: the membranes were placed into six individual wells of a 96-well titer plate; two wells comprised membrane/HEL-11-25; two wells comprised membrane/HEL-106-116; and two wells comprised

25   membrane. The following conditions were utilized for each well. A solution of 1% BSA was added to each well, followed by room temperature incubation for 1 hr. This was followed by 3 x 250μl washings with phosphate-buffered saline ("PBS"). The anti-HEL-11-16 was added to

30   one well from each set and the anti-HEL-106-116 was added to one well from the remaining set. Thereafter, 100μl of goat-anti-mouse antibody conjugated with alkaline phosphatase (1:5000 dilution) (High Clone, Utah, Part #EA 1055-X) was added to each well. This was followed by

35   room temperature incubation for 30min; thereafter, 3 x 250μl PBS washings were conducted. Afterwards, 100μl of NBT-BCIP (nitro blue tetrazolium -Sigma N6876; 5-bromo-4-

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chloro-3-indolyl-phosphate- Sigma, B6149 prepared as
follows: 66μl of NBT stock (0.5g of NBT in 10ml of
dimethylformamide) and 33μl of BCIP stock (0.5g of BCIP
in 10ml of 70% dimethylformamide) added to 10ml of
5   alkaline phosphatase buffer (100mm NaCl; 5mm MgCl$_2$; 100mm
tris-hydroxymethyl aminomethane, pH 9.5)) solution was
added to each well. A 10min period for color (blue)
development was allotted. Thereafter, the wells were
washed with deionized water. The presence of blue color
10   following the washing indicated the presence of goat-anti
mouse bound to the monoclonal antibodies (results not
presented).

The respective antibodies bound specifically to
15   their respective peptides. E.g., no blue color was
observed in the wells comprising membrane/HEL-11-16 and
aminated polypropylene to which was added anti-HEL-106-
116. No color in the aminated polypropylene indicates
that non-specific binding of either antibody did not
20   occur. The specific binding of the antibodies to
specific peptides indicates, <u>inter</u> <u>alia</u>, that the HEL-11-
25 and HEL-106-116 peptides were indeed correctly
synthesized onto the aminated polypropylene.

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EXAMPLE VI
Dipstick Hybridization

5   Aminated polypropylene film having SAM 125
covalently attached thereto was soaked for 10min in
Hybridization Buffer. Thereafter, $3\mu l$ 615 Fluorescent
Probe in $97\mu l$ Hybridization Buffer ($100pmol/100\mu l$) was
added thereto, followed by incubation for 90min. This
10   was followed by 4 x $200\mu l$ washings with Hybridization
Buffer. The film was then removed and placed onto a
glass slide, followed by analysis using the CCD camera; a
laser-printer reproduction of the results are presented
in FIG. 5
15

       The results of FIG. 5 indicate that strong
hybridization occured between SAM 125 and 615 Flourescent
Probe. The length of the probe vis-a-vis the target did
not interfere with hybridization. This dip-stick format
20   allows for rapid analysis for the presence or absence of
a genetic mutation of interest; the use of non-radiactive
labels avoids certain concerns raised by the use of
radioactive labels.

25   While the foregoing has been described in
considerable detail and in terms of preferred
embodiments, these are not to be construed as limitations
on the disclosure or claims to follow. Modifications and
changes that are within the purview of those skilled in
30   the art are intended to fall within the scope of the
following claims.

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SEQUENCE LISTING

(1)   GENERAL INFORMATION

5

(i)   APPLICANT: Coassin, Peter J.
                      Matson, Robert S.
                      Rampal, Jang B.

10   (ii)   TITLE OF INVENTION: Biopolymer Synthesis
                                      Utilizing Surface
                                      Activated, Organic
                                      Polymers

15   (iii)   NUMBER OF SEQUENCES: 15

        (iv)   CORRESPONDENCE ADDRESS:

                (A)   ADDRESSEE: Beckman Instruments, Inc.
20   (B)   STREET: 2500 Harbor Boulevard
                (C)   CITY: Fullerton
                (D)   STATE: California
                (E)   COUNTRY: USA
                (F)   ZIP: 92634

25
        (v)   COMPUTER READABLE FORM:

                (A)   MEDIUM TYPE: Diskette, 3.5 inch,
                                       1.44 Mb
30   (B)   COMPUTER: IBM
                (C)   OPERATING SYSTEM: MS.DOS
                (D)   SOFTWARE: WordPerfect 5.1

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(vi)   CURRENT APPLICATION DATA:

          (A)   APPLICATION NUMBER:
          (B)   FILING DATE:
5   (C)   CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

          (A)   NAME: Burgoon, Richard P.
10   (B)   REGISTRATION NUMBER: 34,787
          (C)   REFERENCE/DOCKET NUMBER: 129D-141

(ix)   TELECOMMUNICATION INFORMATION:

15   (A)   TELEPHONE: (714) 773-6969
          (B)   TELEFAX: (714) 773-7936

(2)   INFORMATION FOR SEQ ID NO: 1:

20   (i)   SEQUENCE CHARACTERISTICS:

          (A)   LENGTH: 16 bases
          (B)   TYPE: nucleic acid
          (C)   STRANDEDNESS: single
25   (D)   TOPOLOGY: linear

(ii)   MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: no
30

(iv)   ANTI-SENSE: no

(xi)   SEQUENCE DESCRIPTION: SEQ ID NO:1:

35   CAA CAT TTC GGT TGT G   16

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(3)   INFORMATION FOR SEQ ID NO: 2:

       (i)   SEQUENCE CHARACTERISTICS:

5   (A)   LENGTH: 16 bases
                      (B)   TYPE: nucleic acid
                      (C)   STRANDEDNESS: single
                      (D)   TOPOLOGY: linear

10   (ii)   MOLECULE TYPE: DNA (genomic)

       (iii) HYPOTHETICAL: no

       (iv)   ANTI-SENSE: no

15
       (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGT GTA AAG CCA ACA C   16

20   (4)   INFORMATION FOR SEQ ID NO: 3:

       (i)   SEQUENCE CHARACTERISTICS:

                      (A)   LENGTH: 16 bases
25   (B)   TYPE: nucleic acid
                      (C)   STRANDEDNESS: single
                      (D)   TOPOLOGY: linear

       (ii)   MOLECULE TYPE: DNA (genomic)

30
       (iii) HYPOTHETICAL: no

       (iv)   ANTI-SENSE: no

35

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(xi)   SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGT GTA AGG CCA ACA C   16

5   (5)   INFORMATION FOR SEQ ID NO: 4:

(i)   SEQUENCE CHARACTERISTICS:

(A)   LENGTH: 14 bases
10   (B)   TYPE: nucleic acid
(C)   STRANDEDNESS: single, including nick
(D)   TOPOLOGY: linear

(ii)   MOLECULE TYPE: DNA (genomic)
15

(iii)   HYPOTHETICAL: no

(iv)   ANTI-SENSE: no

20   (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGT GTA AAC AAC AC   14

(6)   INFORMATION FOR SEQ ID NO: 5:
25

(i)   SEQUENCE CHARACTERISTICS:

(A)   LENGTH: 16 bases
(B)   TYPE: nucleic acid
30   (C)   STRANDEDNESS: single
(D)   TOPOLOGY: linear

(ii)   MOLECULE TYPE: DNA (genomic)

35   (iii)   HYPOTHETICAL: no

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(iv)　ANTI-SENSE:　no

(xi)　SEQUENCE DESCRIPTION:　SEQ ID NO:5:

5　　　T TTA TAG TAG AAA CCA　　　　　　　　　　　　　　16

(7)　INFORMATION FOR SEQ ID NO: 6:

(i)　SEQUENCE CHARACTERISTICS:

10

(A)　LENGTH:　16 bases
(B)　TYPE:　nucleic acid
(C)　STRANDEDNESS:　single
(D)　TOPOLOGY:　linear

15

(ii)　MOLECULE TYPE:　DNA (genomic)

(iii)　HYPOTHETICAL:　no

20　　　(iv)　ANTI-SENSE:　no

(xi)　SEQUENCE DESCRIPTION:　SEQ ID NO:6:

T TCT TTT ATA GTA GAA　　　　　　　　　　　　16
25

(8)　INFORMATION FOR SEQ ID NO: 7:

(i)　SEQUENCE CHARACTERISTICS:

30　　　　　　(A)　LENGTH:　98 bases
(B)　TYPE:　nucleic acid
(C)　STRANDEDNESS:　single
(D)　TOPOLOGY:　linear

35　　　(ii)　MOLECULE TYPE:　DNA (genomic)

      (iii) HYPOTHETICAL: no

      (iv)   ANTI-SENSE: no

5   (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
G TTT TCC TGG ATT ATG CCT GGC ACC ATT                    28
AAA GAA AAT ATC ATC TTT GGT GTT TCC                      55
TAT GAT GAA TAT AGA TAC AGA AGC GTC                      82
ATC AAA GCA TGC CAA                                      98
```

  (9)   INFORMATION FOR SEQ ID NO: 8:

      (i)   SEQUENCE CHARACTERISTICS:

          (A)   LENGTH: 95 bases
          (B)   TYPE: nucleic acid
          (C)   STRANDEDNESS: single
          (D)   TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

      (iii) HYPOTHETICAL: no

      (iv) ANTI-SENSE: no

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
G TTT TCC TGG ATT ATG CCT GGC ACC ATT                    28
AAA GAA AAT ATC ATT GGT GTT TCC TAT                      55
GAT GAA TAT AGA TAC AGA AGC GTC ATC                      82
AAA GCA TGC CAA C                                        95
```

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(10)   INFORMATION FOR SEQ ID NO: 9:

(i)   SEQUENCE CHARACTERISTICS:

5   (A)   LENGTH: 24 bases
                 (B)   TYPE: nucleic acid
                 (C)   STRANDEDNESS: single
                 (D)   TOPOLOGY: linear

10   (ii)   MOLECULE TYPE: DNA (genomic)

         (iii)   HYPOTHETICAL: no

         (iv)   ANTI-SENSE: no
15

         (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:9:

         G TTT TCC TGG ATT ATG CCT GGC AC   24

20   (11)   INFORMATION FOR SEQ ID NO: 10:

         (i)   SEQUENCE CHARACTERISTICS:

                 (A)   LENGTH: 24 bases
25   (B)   TYPE: nucleic acid
                 (C)   STRANDEDNESS: single
                 (D)   TOPOLOGY: linear

         (ii)   MOLECULE TYPE: DNA (genomic)
30
         (iii)   HYPOTHETICAL: no

         (iv)   ANTI-SENSE: yes

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(xi)   SEQUENCE DESCRIPTION: SEQ ID NO:10:

G TTG GCA TGC TTT GAT GAC GCT TC   24

5   (12)   INFORMATION FOR SEQ ID NO: 11:

       (i)   SEQUENCE CHARACTERISTICS:

                (A)   LENGTH: 17 bases
10   (B)   TYPE: nucleic acid
                (C)   STRANDEDNESS: single
                (D)   TOPOLOGY: linear

       (ii)   MOLECULE TYPE: DNA (genomic)
15
       (iii)   HYPOTHETICAL: no

       (iv)   ANTI-SENSE: no

20   (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCA GCT ACC GTA AAT GT   17

       (13)   INFORMATION FOR SEQ ID NO: 12:
25
       (i)   SEQUENCE CHARACTERISTICS:

                (A)   LENGTH: 15 amino acids
                (B)   TYPE: amino acids
30   (C)   STRANDEDNESS: single
                (D)   TOPOLOGY: linear

       (ii)   MOLECULAR TYPE: Peptide
       (iii)   HYPOTHETICAL: no
35

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(xi)   SEQUENCE DESCRIPTION: SEQ ID NO:12:

         Ala Met Lys Arg His Gly Leu   7
         Asp Asn Tyr Arg Gly Tyr Ser Leu   15

5

   (14) INFORMATION FOR SEQ ID NO: 13:

      (i)   SEQUENCE CHARACTERISTICS:

10   (A)   LENGTH: 11 amino acids
                 (B)   TYPE: amino acids
                 (C)   STRANDEDNESS: single
                 (D)   TOPOLOGY: linear

15   (ii)   MOLECULAR TYPE: Peptide
      (iii) HYPOTHETICAL: no
      (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:13:

         Asn Ala Trp Val Ala Trp   6
20   Arg Asn Arg Cys Lys   11

   (15) INFORMATION FOR SEQ ID NO: 14:

      (i)   SEQUENCE CHARACTERISTICS:

25
                 (A)   LENGTH: 15 bases
                 (B)   TYPE: nucleic acid
                 (C)   STRANDEDNESS: single
                 (D)   TOPOLOGY: linear

30
      (ii)   MOLECULE TYPE: DNA (genomic)

      (iii) HYPOTHETICAL: no

35   (iv)   ANTI-SENSE: no

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(xi)   SEQUENCE DESCRIPTION: SEQ ID NO:14:

AAG GAC CTA ATA CGG   15

5   (16)   INFORMATION FOR SEQ ID NO: 15:

(i)   SEQUENCE CHARACTERISTICS:

(A)   LENGTH: 24 bases
10   (B)   TYPE: nucleic acid
(C)   STRANDEDNESS: single
(D)   TOPOLOGY: linear

(ii)   MOLECULE TYPE: DNA (genomic)
15

(iii)   HYPOTHETICAL: no

(iv)   ANTI-SENSE: no

20   (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTT TTC CTG GAT TAT GCC TGG CAC   24

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CLAIMS

What is claimed is:

5   1.   In a method for synthesizing biopolymers or biomonomers onto a solid support material, the improvement comprising use of an organic polymer comprising nucleophiles adsorbed to the surface of the polymer.

10

2.   The method of claim 1 wherein the polymer is selected from the group consisting of polypropylene, polyethylene, polybutylene, polyisobutylene, polybutadiene, polyisoprene, polyvinylpyrrolidone,
15   polytetrafluoroethylene, polyvinylidene difluoride, polyfluoroethylene-propylene, polyethylenevinyl alcohol, polymethylpentene, polychlorotrifluoroethylene, polysulfones, and blends or copolymers thereof.

20   3.   The method of claim 1 wherein the nucleophile is selected from the group consisiting of amine, hydroxyl, thiol, carboxylate and substituents comprising at least one of the foregoing.

25   4.   The method of claim 1 wherein the biopolymer or biomonomer is selected from the group consisting of nucleic acids, oligonucleotides, amino acids, proteins, peptides, hormones, oligosaccharides, lipids, glycolipids, lipopolysaccharides, phospholipids,
30   inverted nucleotides, peptide nucleic acids, meta-DNA, avidin, biotin, antibodies, analogues of the foregoing, and combinations of at least two of the foregoing.

5.   The method of claim 1 wherein the polymer
35   is polypropylene.

6.   The method of claim 1 wherein the surface
of the polymer comprises an amine.

7.   The method of claim 1 wherein the surface
of the polymer comprise an hydroxyl.

8.   The method of claim 1 wherein the surface
of the polymer comprise a thiol.

9.   The method of claim 1 wherein the
biopolymer is selected from the group consisting of
oligonucleotides, peptides and oligosaccharides.

10.   In a method of synthesizing biopolymers or
biomonomers onto a solid support material, the
improvement comprising use of a solid support selected
from the group consisting of aminated polypropylene,
hydroxylated polypropylene and thiolated polypropylene.

11.   The method of claim 10 wherein the
biopolymer is selected from the group consisting of
oligonucleotides, peptides and oligosaccharides.

12.   In a method for the sequential analysis of
a protein or a peptide, the improvement comprising
attaching the protein or peptide to a nucleophile
adsorbed to the surface of an organic polymer.

13.   The method of claim 12 wherein the polymer
is selected from the group consisting of polypropylene,
polyethylene, polybutylene, polyisobutylene,
polybutadiene, polyisoprene, polyvinylpyrrolidone,
polytetrafluoroethylene, polyvinylidene difluoride,
polyfluoroethylene-propylene, polyethylenevinyl alcohol,

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polymethylpentene, polychlorotrifluoroethylene,
polysulfones, and blends or copolymers thereof.

5   14.   The method of claim 12 wherein the
nucleophile is selected from the group consisiting of
amine, hydroxyl, thiol, carboxylate and substituents
comprising at least one of the foregoing.

         15.   The method of claim 12 wherein the polymer
10   is polypropylene.

         16.   An organic polymer comprising nucleophiles
adsorbed onto the surface thereof wherein at least one
oligonucleotide having a defined sequence is covalently
15   attached to the polymer via the nucleophile.

         17.   The device of claim 16 wherein said
polymer is selected from the group consisting of
polypropylene, polyethylene, polybutylene,
20   polyisobutylene, polybutadiene, polyisoprene,
polyvinylpyrrolidone, polytetrafluoroethylene,
polyvinylidene difluoride, polyfluoroethylene-propylene,
polyethylenevinyl alcohol, polymethylpentene,
polychlorotrifluoroethylene, polysulfones, and blends or
25   copolymers thereof.

         18.   The device of claim 16 wherein said
nucleophile is selected from the group consisiting of
amine, hydroxyl, thiol, carboxylate and substituents
30   comprising at least one of the foregoing.

         19.   The device of claim 16 wherein the polymer
is selected from the group consisting of hydroxylated
polypropylene, thiolated polypropylene, and aminated
35   polypropylene.

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20.   The device of claim 16 wherein the length of said oligonucleotide is about 250 nucleotides.

21.   The device of claim 16 wherein the oligonucleotide is complementary to the sequence of a gene of interest.

22.   A method of analyzing a gene of interest comprising the steps of:

a.   obtaining a sample comprising a gene of interest;

b.   introducing said sample to the device of claim 21; and

c.   determining if said gene has hybridized in a complementary manner to the oligonucleotide of the device of claim 21.
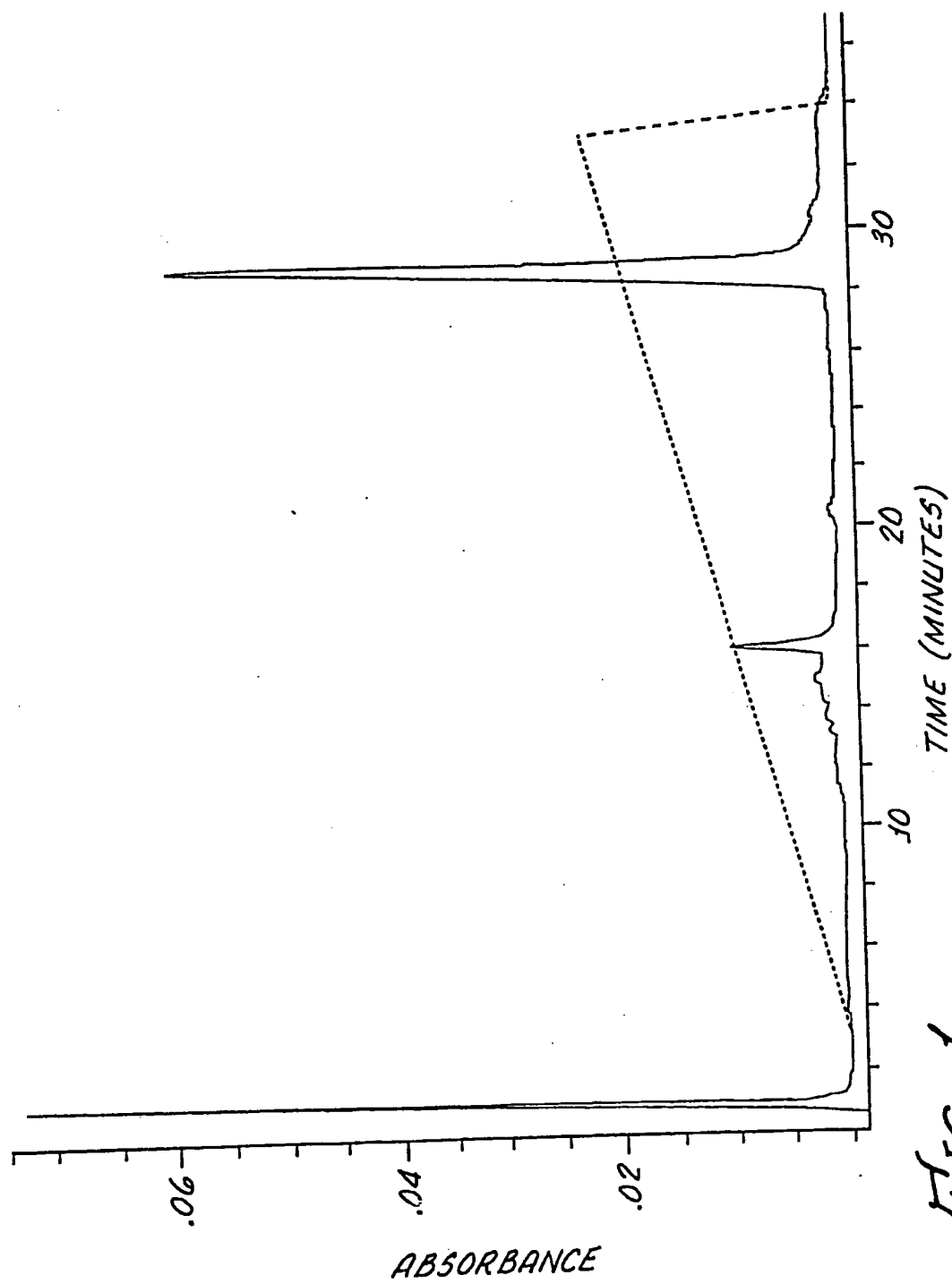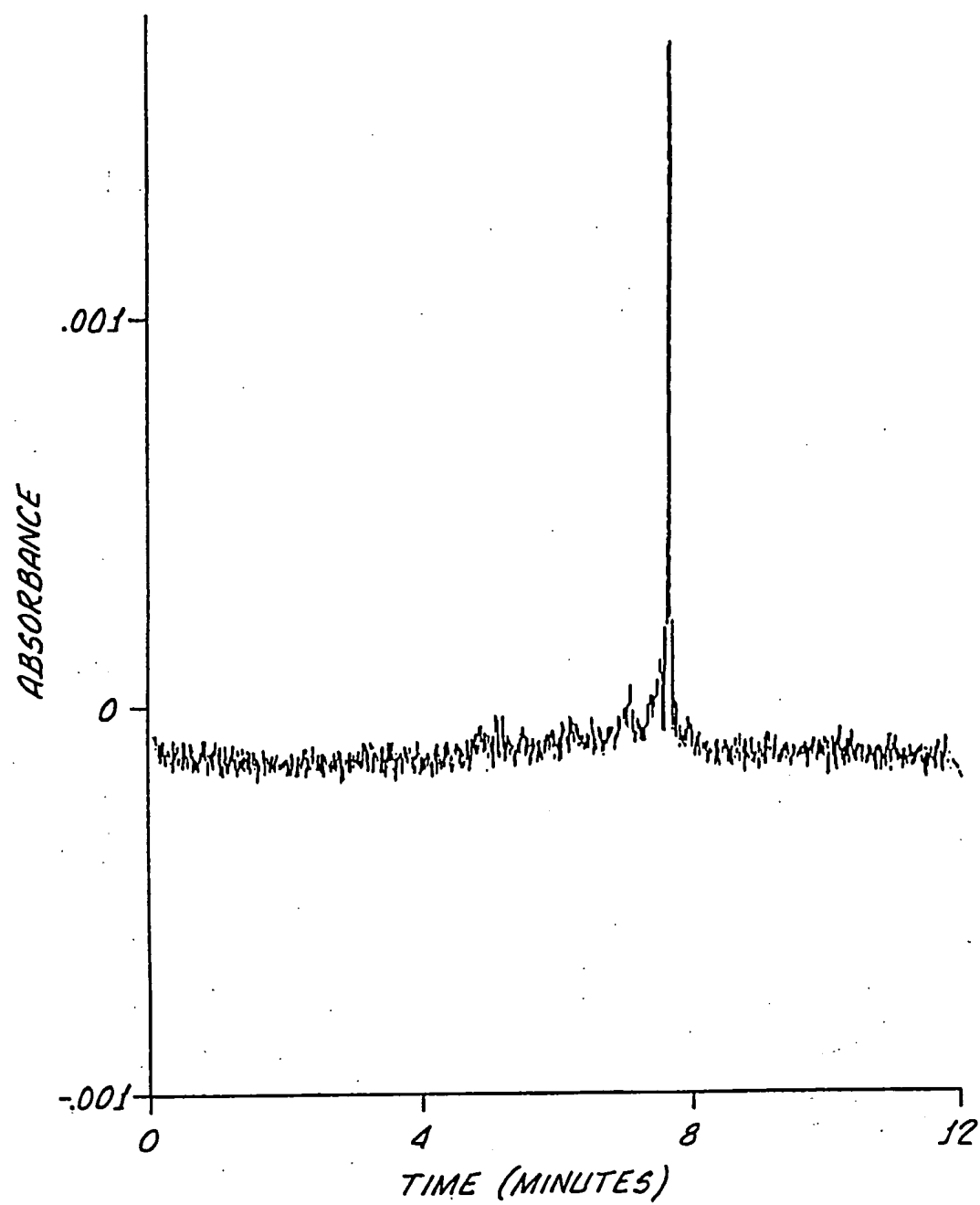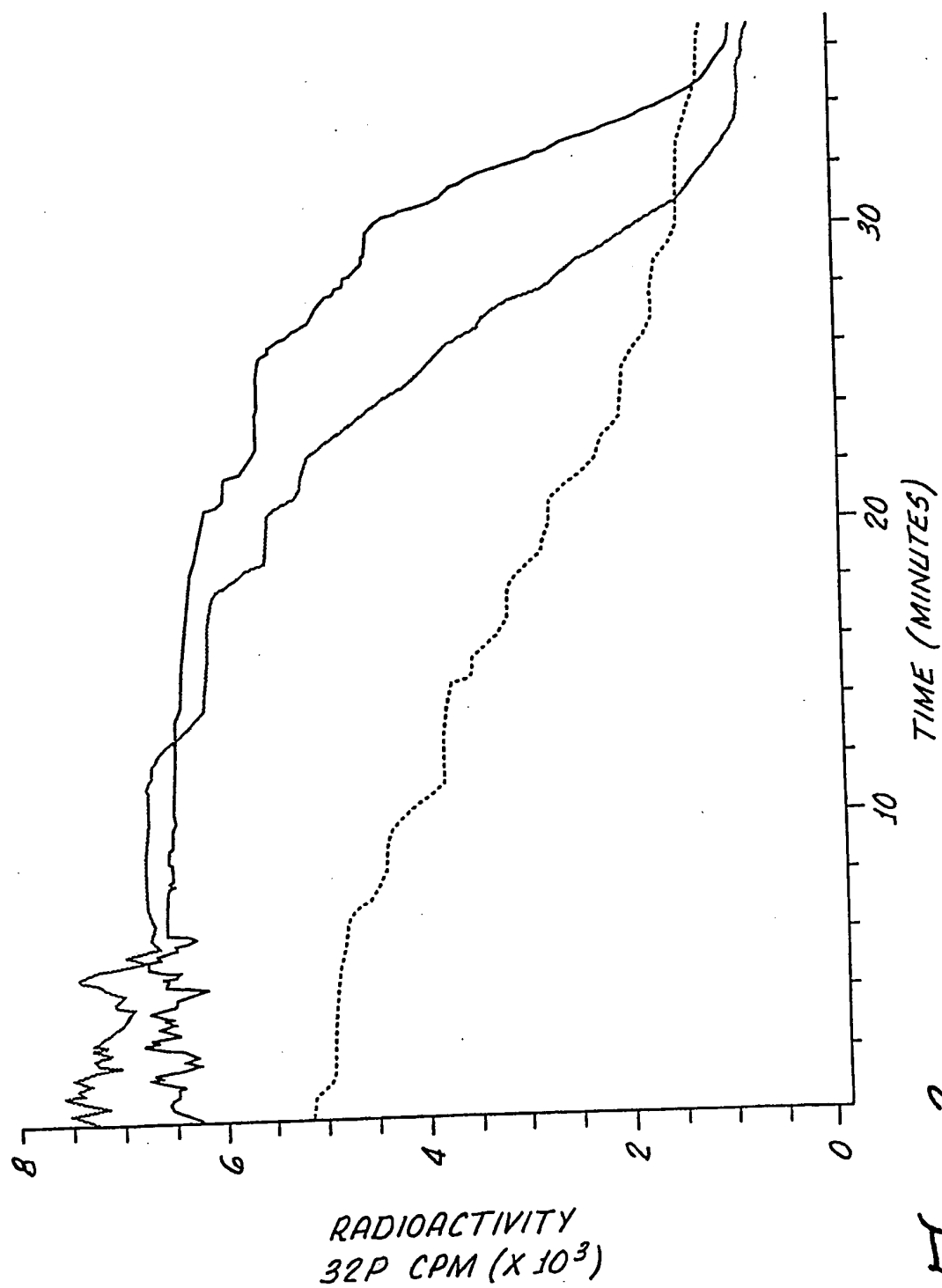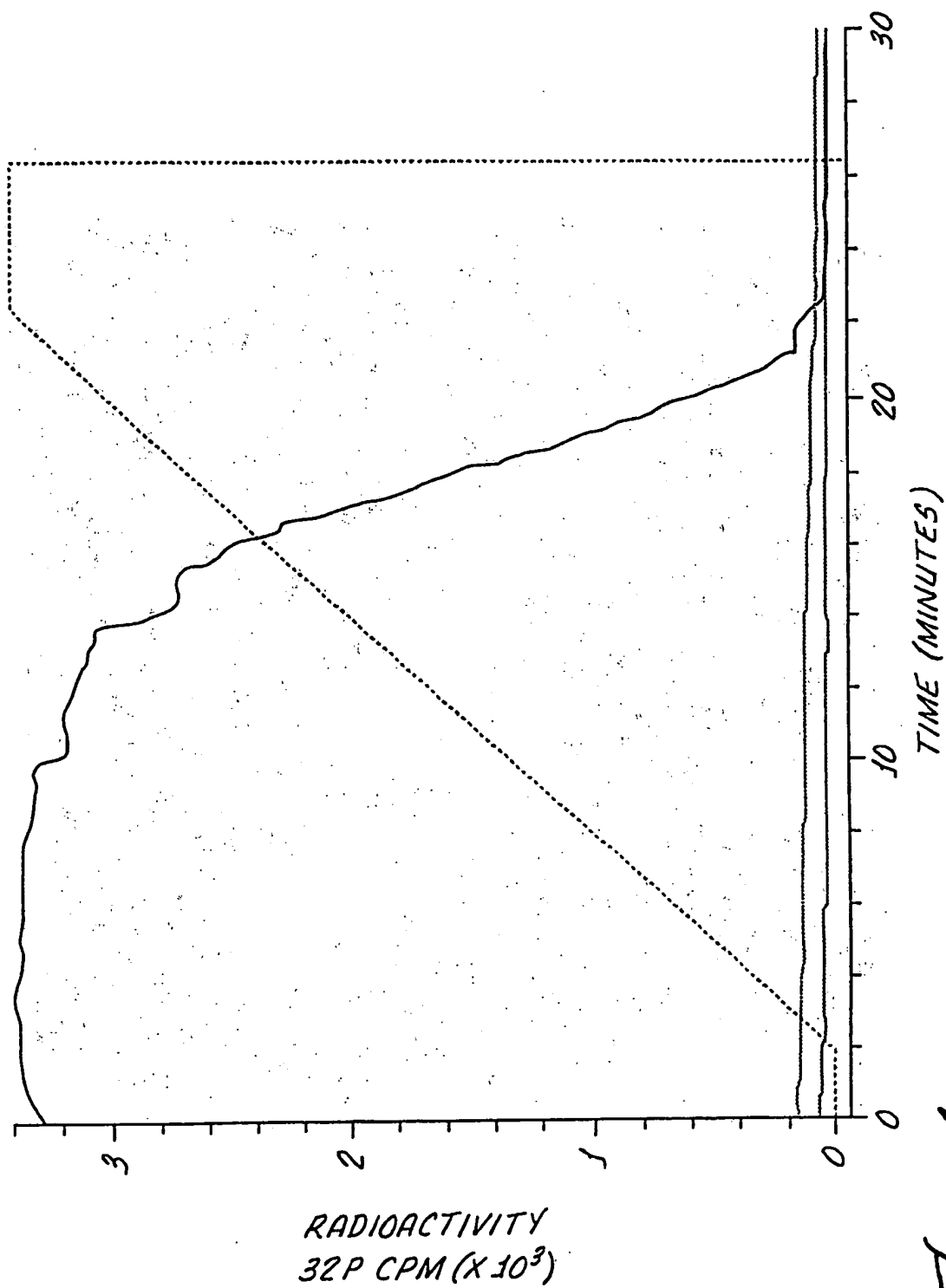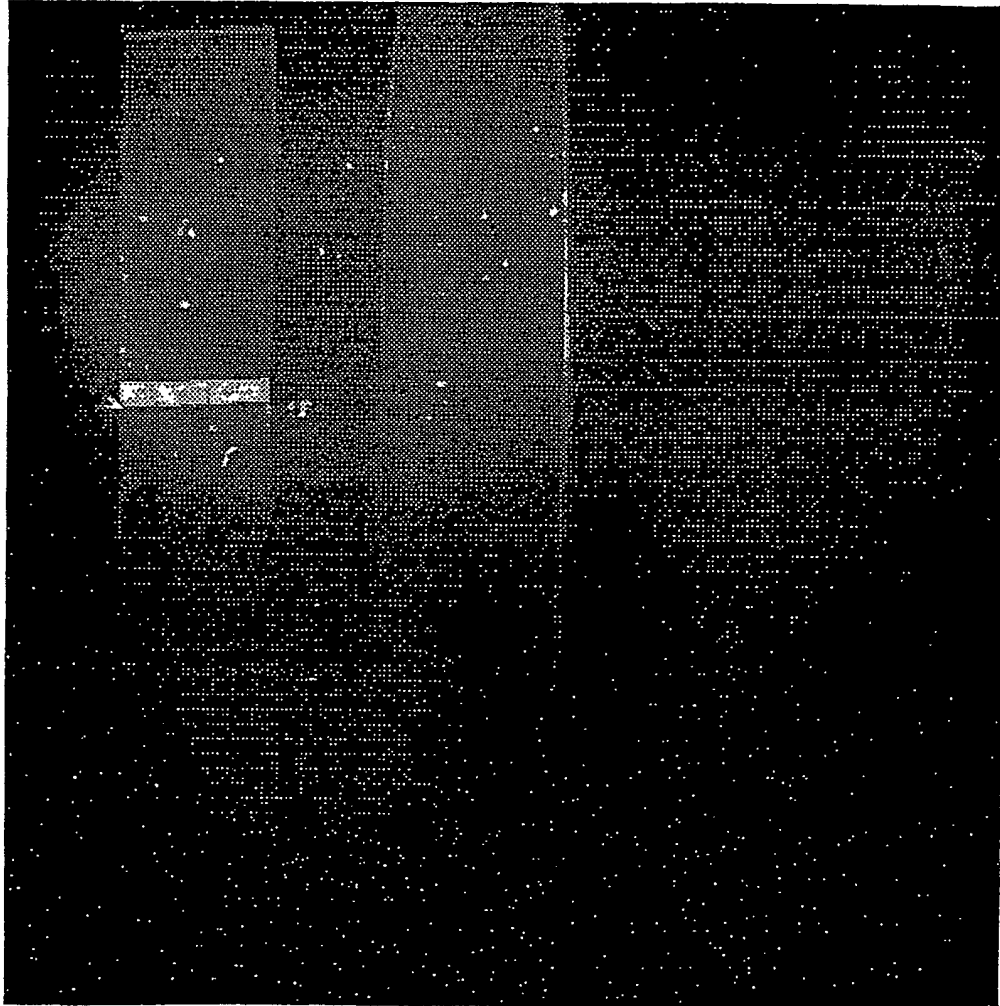
Fig. 1.

FIG. 2.

Fig. 3.

FIG. 4.

FIG. 5.

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   C07H21/00   C12Q1/68   C07K1/04   G01N33/68   C08F8/30
         C08J3/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   C07H   C07K   C12Q   G01N   C08F   C08J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 319 957 (GLUETECH APS) 14 June 1989 see page 5, line 49 - line 54 ---	1,16,22
A	EP,A,0 305 929 (MILLIPORE CORPORATION) 8 March 1989 see page 4, line 30 - page 5, line 15 & US,A,4 923 901 cited in the application ---	1,16,22
A	WO,A,90 15883 (UNIVERSITY OF UTAH) 27 December 1990 see page 6, line 26 - page 11, line 13 ---	1
A	WO,A,91 13098 (FORSKNINGSCENTER RISO) 5 September 1991 see examples -----	1

☐ Further documents are listed in the continuation of box C.   ☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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